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DAVID BODIAN 1

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THE VIRUS, THE NERVE CELL, AND PARALYSIS

A Study of Experimental Poliomyelitis in the Spinal Cord[1]

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Received for Publication March 5, 1948

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INTRODUCTION

Published observations on the histopathological changes in the spinal cord in poliomyelitis are so numerous that it would seem little can be added to what has been reported. The complexity of the problems of pathogenesis, however, has resulted in contradictory interpretations of certain findings, and has left many points unsettled. It was felt that some of the important points remaining at issue perhaps could be investigated more satisfactorily by means of an approach which attempts to correlate pathological processes in chronological sequence, with changes in level of virus activity on the one hand and changes in clinical function on the other. It was felt that such an approach requires at least approximate quantitative data at all three levels of observation, and could therefore be essayed only in the experimental animal. The findings and interpretations to be reported are by no means complete, and because of the nature of the material the quantitation of the variables involved often leaves much to be desired. It is hoped, nevertheless, that the results may be useful as a rough scaf-

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folding for the tasks of planning and interpretation of studies at the chemical or virus level, and of improving our understanding of cellular changes in relation to paralysis and recovery. The basic question of the time sequence of rise and fall of virus concentration in the infected rhesus spinal cord has not received close attention except by Brodie ('33) and Bodian and Cumberland ('47). The findings in these studies have served as an important background for orientation in the work reported here.

The first objective of this study was to determine the sequence of histological changes which occurs in the spinal cord from the period of onset of pathological changes to the period of functional recovery in poliomyelitis, and to establish relatively constant features of the cytopathological process in quantitative detail. The changes in the motoneuron population were studied particularly, since the motoneurons bear the brunt of the pathological effects and probably constitute the principal source of substrate for virus proliferation. In addition, their destruction is the principal cause of motor paralysis and of muscle atrophy which follows the paralysis. The population unit selected for study was the motoneuron group supplying a single extremity, arm or leg, and consisting of about 14,000 motor nerve cells. This population unit was selected because it can be identified spatially much more accurately than smaller units supplying individual muscles or muscle groups, because it is relatively compact in distribution, and because the field it supplies, the arm or leg, is a functional unit not too difficult to assess in terms of degree of functional loss. In fact, therefore, the motoneuron population supplying a limb, plus the limb itself, can be considered as a model for the disease in the whole organism, undergoing the characteristic ebb and flow of the disease processes in all their ramifications.

Although important contributions to an understanding of the pathological events of the acute stage, and especially the preparalytic period, have been made by Hurst ('29) and by Pette, Demme, and Környey ('32) in their experimental studies, quantitative studies of the sequence of cellular changes in a close series of infective stages have been made only by Covell ('32). In his excellent study, unfortunately, Covell failed to appreciate that changes in cytoplasmic Nissl substance were sufficiently distinctive so that they could be used as a quantitative

measure of both the degree of injury and of recovery of nerve cells. Moreover, as he was aware, degenerative changes were overemphasized in his analysis, since all his animals had the prostrating type of paralysis produced by the Rockefeller Institute MV virus.

MATERIAL

Rhesus monkeys were used to enable study of changes from the earliest period of pathological effect to late stages of recovery, and to permit more satisfactory fixation and staining of tissues than is possible in human material. In all, some 50 rhesus monkeys killed from a few days to one year after inoculation with several "strains" of virus of recent human origin were studied quantitatively, and many others were available for qualitative comparisons. These monkeys had been examined in considerable clinical detail in most cases before they were killed, to determine the time of onset of symptoms, the degree of weakness, and the extent of recovery. Motor function of animals was determined by inspection of running, jumping and climbing performance in large cages, by hand examination of individual extremities to determine asymmetry of power of flexion and extension at joints, and by palpation to detect abnormalities of tone and atrophy. In most cases daily examinations were made during the acute period of the disease, with weekly, and later monthly, examinations during the convalescent period. In some animals, voluntary muscle performance was compared with the response to faradic stimulation of nerve points and exposed peripheral nerves at the time of autopsy.

After final clinical examination, animals were anesthetized with ether, and perfused through the aorta with 50 ml. of normal saline followed by 1 to 2 liters of 10% formol containing 1% acetic acid. Some animals were fixed with 10% neutral formol, or with Regaud's fluid, for better study of mitochondria and inflammatory changes. The fixation of tissues by vascular perfusion of the living anesthetized animal was of great value in eliminating most of the postmortem artifacts seen in material fixed with the usual immersion technique. The cervical and lumbosacral spinal cord enlargements were removed carefully, and the four segments contributing principally to the innervation of upper and lower extremities were prepared for paraffin embedding. In the cervical region the cord segments regularly sectioned

were the 6th, 7th, and 8th cervical, and the 1st thoracic (C6, C7, C8, T1); in the lumbosacral region the 4th, 5th, 6th, and 7th lumbar (L4, L5, L6, L7). When the lumbar plexus was pre- or post-fixed, the four segments which had the largest spinal roots were considered for formal purposes as the 4th to 7th lumbar. Complete serial sections in paraffin were cut at 15μ and stained with gallocyanin, according to Einarson's method ('32), or alternately with gallocyanin, haematoxylin-eosin-azure, and activated protargol (Bodian, '36). Mitochondria were studied in 7μ sections of Regaud material, stained with iron-alum haematoxylin.

Serial sections of spinal cords from 10 human cases and 4 chimpanzee cases of poliomyelitis were available for comparison. Material from monkeys infected with Western equine encephalomyelitis virus, and with neurotropic yellow fever virus (17D strain), was also used for certain comparative purposes.

PLAN OF STUDY

A preliminary survey of all the material available, including chimpanzee and human spinal cords, established the essential similarity of the pathological process in all three primate species during the period of progressive paralysis and later. The earliest changes of the pre-paralytic period were available only in the rhesus monkey.

Attention was limited to the changes in the lateral cell columns of the anterior horn of the spinal cord of the brachial and lumbar enlargements, so that histopathological events might be correlated in time and in degree with changes in muscle function of each extremity. It was felt that only in this way was it possible to deal with the dynamics of the disease process. Because of the mixture of stages of cell change found in any one specimen, it was necessary to establish the precise sequence of events by statistical means, so that much time was devoted to this end. As a routine, 50 to 60 sections, out of a total of about 1200 sections in each brachial or lumbar enlargement, were subjected to cell counts. These sections were regularly spaced throughout the cord segments involved, so that 10 to 15 sections were examined as a sample of each of the four principal segments contributing to the brachial and lumbar plexuses, respectively. These segments in the brachial region are C6 to T1 inclusive, and in the lumbar region L4

to L7, inclusive. Although C5, T2, and S1 contribute to their respective limb plexuses, they were not usually studied. It was felt that the status of the motoneuron population of the four principal segments was an adequate indicator of the function of the innervated limb, so that study of the marginal segments would add tedium without much increase of accuracy. In all material only cells containing a recognizable nucleus and nucleolus were counted.

DESCRIPTION AND DEFINITION OF CLINICAL STAGES

For the purposes of this study it was necessary to define carefully the clinical stages of the disease in experimental animals, so that accurate correlations could be made between the clinical events, histopathological changes, and the rise and fall of virus in infected nervous tissue. Table 1 indicates the stages with extremes of time range observed in animals in this series. Other animals in this laboratory have exhibited incubation periods as short as 3 days and as long as 54 days. The *incubation stage* will be defined as the phase following inoculation and preceding clinical signs. This phase is subdivided into a *latent period* during which no virus or lesions can be detected in the central nervous system (CNS), and an early preparalytic or *presymptomatic period*, during which no symptoms are apparent, but during which virus and cell changes can be found in the CNS near the point of inoculation. The onset of fever, when it occurs, opens the following, or *acute stage*, beginning with the late preparalytic period. This period precedes detectable flaccid paralysis, and is characterized by generalized symptoms such as fever, tremor, spasticity and ataxia (Romer, '11; Harmon, et al., '31; Pette, et al., '32; Bodian and Cumberland, '47).

In the experimental animal, as in man, symptoms may or may not be present preceding paralysis. With the virus strains generally employed in experimental work, the preparalytic period usually covers an interval of about one day, but not infrequently two or three days. With small doses of virus, or with "mild" strains, the preparalytic period may be prolonged to five or more days, during which period fever, tremor, and clasp-knife spasticity may be present in varying degrees. For purposes of definition, it is to be noted that, irrespective of its duration, the preparalytic period opens with the critical phase of

beginning virus activity. It is obvious that the termination of this period is arbitrarily defined. Since extensive lesions of anterior horn cells may occur before muscle weakness is clinically recognized, the onset of the *period of increasing paralysis* is not sharply demarcated. In terms of pathological histology, moreover, the latter period is simply

TABLE 1

Stages of Poliomyelitis in Rhesus Monkeys

	DURATION IN DAYS		CLINICAL SYMPTOMS	VIRUS	PATH. CHANGES
	Mean	Range			
Incubation Stage					
Latent Period	8	3 to 22	0	0	0
Early Preparalytic Period	1		0	+	+
Acute Stage (febrile)					
Late Preparalytic Period	3	1 to 5	+	+ to 4+	+
Period of Increasing Paralysis	3	1 to 5	+	4+ to +	+ to 4+
Subacute Stage (Early Recovery Period)	21	15 to 40	+	+	+
Convalescent Stage (Period of Continued Recovery)	60	30 to 300	+	−	+
Chronic Stage (No Further Recovery)					

TABLE 2

Time Course of Paralysis, Atrophy, and Recovery in Rhesus Monkeys

	PERIOD OF DISEASE AFTER ONSET OF PARALYSIS	
	Mild Cases	Severe Cases
Height of Paralysis	1 to 3 days	2 to 5 days
Onset of Atrophy		2 to 3 weeks
Beginning Recovery of Power	1 to 3 weeks	1 to 2 months
Limit of Increase of Power and Muscle Size	1 week to 3 months	3 months to 1 year

a continuation of the preceding one, so that both periods represent the phase of progressive degradation of nerve cell functions due to virus activity.

The *period of increasing paralysis* is followed by a *period of early recovery*, which, according to pathological criteria, is a phase of neuron recovery, and is usually largely completed about three to six weeks after onset of paralysis. After this time, remaining nerve cells in the spinal

cord are essentially normal in appearance in all cases, as Hurst ('29) found in his single recovered animal, so that further functional recovery must be referable to other recovery and compensation changes in the CNS and in muscle. These changes occur in the *convalescent stage* and are followed by a phase of no further recovery, the *chronic stage*. Table 2 shows the time course of some of the critical points in the sequence of paralysis, atrophy, and recovery.

In defining the stages of the disease, an attempt has been made to coordinate clinical and pathological findings, since they are part of a single process. As is well known, the disease in man follows a rather similar course, and the usually described clinical stages are comparable with those observed in the monkey, except for minor differences in time course of stages. For example, the stage of convalescence is probably of shorter duration, as a rule, in the monkey as compared with man.

DESCRIPTION AND DEFINITION OF GRADES OF PARALYSIS

Limbs were graded as to degree of loss of voluntary muscle power, with flaccidity, hereafter termed weakness or paralysis. Six categories were established arbitrarily, with 5 or "Normal" representing one extreme and 0 representing the other, that of complete flaccid paralysis. It was found possible, with the method of grading used, to obtain excellent agreement upon independent observations by the same or different examiners. The grading for purposes of this report is defined as follows:

5—"Normal".
4—Definite weakness, but capable of using limb effectively in climbing, running, or jumping.
3—Uses limb poorly in climbing, running, or jumping.
2—Barely able to use limb in climbing or running movements, but able to move limb segments readily against gravity.
1—Dangling limb with only feeble movements at skeletal joints.
0—Complete flaccid paralysis, or only faint traces of movement.

All the gradings were made with regard to maximal possible effort, and with reference to normal function preceding the onset of paralysis. Since individual animals vary greatly in normal strength depending on size and physical condition, any grading more closely spaced could

hardly be comparable from animal to animal, or accurate. The above six gradings roughly correspond to those used by Kristensen and Wulff ('47) for human cases, and follow as closely as possible the percentage grading used by Lenhard and the Kendalls in their human cases ('43). In animals in poor physical condition, even these grades cannot be applied with confidence by an experienced examiner, but in healthy, vigorous animals it was often felt that closer grading could have been used at times. This was especially true in comparing right and left arms or legs, when one member of a pair was slightly weaker than the other. It may be said, in anticipation of the more detailed analysis to follow, that in animals later shown by histopathological study to be definitely poliomyelitic, clinical estimates of muscle power were correlated well with the severity of lesions in anterior horns. In no case was an extremity rated weaker than another in the same animal without a correspondingly greater amount of motoneuron damage in its respective anterior horn. This indicates that a correlation does exist between severity of paralysis and the degree of motoneuron damage, and presently it will be seen how closely correlated these functions are.

DESCRIPTION OF FINDINGS

I. Cytopathological Changes in Motor Nerve Cells

The fundamental concept that the primary injury in poliomyelitis occurs in the anterior horn cells, and is the cause of the motor paralysis, was first stated by Charcot (1870). The reasonable objection that his conclusions were gratuitous because based on chronic cases was not soon answered. Acute cases showed inflammatory lesions which secondarily could have affected nerve cells. It was Rissler (1888) who first made an adequate study of acute cases and presented evidence that the primary pathological process occurred in nerve cells, and that inflammatory changes were secondary. The controversy which followed Charcot's publication, however, remained active until experimental evidence was presented indicating that neuron changes could occur independently of inflammatory changes in the preparalytic period (Hurst, '29), and that the infiammatory response did not occur in de-neuronated, but susceptible, nerve centers (Bodian and Howe, '41). That the entire pattern of reaction of non-neuronal elements cannot be accounted for on the basis of a secondary response to neuron changes

alone has been apparent to most modern workers. But, regardless of the complex chain of probably interdependent cellular events which follow the onset of the pathological process, it cannot be doubted now that the neurons are involved in the earliest stages of reaction, and that their injury precedes and is independent of inflammatory cell changes. The presumption at the present time is, therefore, that the regressive changes in motoneurons to be described in this section are the result of direct virus action. Most of the regressive changes may be found when virus is first present in detectable concentration in the anterior horns (Bodian and Cumberland, '47).

Nissl Substance. The first microscopic intracellular change seen in motoneurons is the reduction in size and in staining capacity of the Nissl bodies (Hurst, '29; Bodian, '45). This change very largely occurs in diffuse fashion throughout the cytoplasm and is characteristic of most of the abnormal motoneurons seen in the preparalytic period (also Sabin and Ward, '41). The frequency of occurrence of different degrees of severity of such diffuse chromatolysis of Nissl substance is such as to indicate clearly that this chromatolysis is progressive and may lead in some cases to complete disappearance of basophilic cytoplasmic material within a period of hours or less. In one of the earliest preparalytic cases examined (959, Table 3), motoneurons showing mild degrees of diffuse chromatolysis were found in much greater numbers than those showing severe chromatolysis, but this order is reversed in late preparalytic and early paralytic cases. The diffuse chromatolysis of early poliomyelitis is arranged in Plate 1 (figs. 1 to 8) to show the probable sequence of progression of the process in cells selected from a single preparalytic case (B12). Plate 2 (figs. 1 to 8) shows stages in the continuing progression of degenerative changes which are probably irreversible.

Evidence which will be presented in detail in a later section indicates that the diffuse thinning of Nissl bodies is characteristic of a period of about 5 days after onset of the pathological process. However, in the period between 3 and 5 days after onset of paralysis, a new morphological pattern of Nissl bodies in infected cells also appears. This pattern consists of thickened Nissl bodies adjoining the cell and nuclear membranes, with a central area in the cytoplasm relatively free of Nissl bodies. This appearance we shall refer to hereafter as "cen-

tral chromatolysis". After the first week central chromatolysis is the predominant appearance of Nissl bodies in injured neurons which are not obviously necrotic. Such cells were described by Sabin and Ward ('41) as cells with marginated Nissl substance, which they observed in cases past the acute stage. It will be shown later in this report that central chromatolysis is a manifestation of the recovery process in nerve cells, and that the reconstitution of Nissl bodies near the cell and nuclear membranes is an important phase of the reparative stage in the motoneurons. A similar process seems to occur in motoneurons regenerating after axon section (Bodian, '47a).

Mitochondria. McCann ('18) appears to have been the first to observe that mitochondria of rhesus motoneurons were apparently unaffected by poliomyelitis virus action even when the Nissl substance had disappeared. Hurst ('29) also found apparently normal mitochondria in neurons in which Nissl substance was already completely absent, and even in some cells which were vacuolated. Mitochondria were absent in necrotic cells, however. Covell ('32) found mitochondria, as well as the Golgi substance, apparently uninvolved in the early stages of poliomyelitis, at a time when chromatolysis, clumping of neutral red granules to one side of the nucleus, and some thickening of neurofibrils was apparent.

Our own findings are in essential agreement with those mentioned above. Mitochondria as seen in iron haematoxylin preparations were found to be normal in appearance in all stages of injury of motoneurons short of acidophilic necrosis (complete loss of basophilia of cytoplasm). This is shown in Plate 3, in which a severely chromatolytic cell (fig. 1) is seen to contain numerous well-stained mitochondria, but a cell in acidophilic necrosis, with severe nuclear changes (fig. 2), contains no demonstrable granules. In fig. 1 the faint outlines of a few remaining Nissl bodies are seen in the cell periphery (above and below).

Since mitochondria are found normal in appearance except in cells showing severe nuclear changes, complete loss of cytoplasmic basophilia, or neuronophagia, it is suggested that dissolution of mitochondria occurs only when cell injury has reached the stage of irreversibility. Although it is easily conceivable that chemical changes in mitochondria, and their functional impairment, may precede visible morphological changes, yet it is difficult to imagine that mitochondrial

functions are directly associated with virus activity. Evidence previously presented (Bodian and Cumberland, '47) indicates that virus concentration reaches high levels in the spinal cord at a time when very few cells are at a stage of cell injury accompanied by mitochondrial changes, but when, and only when, many cells show considerable chromatolysis of Nissl substance. One would ordinarily expect some morphological change of mitochondria at the time of greatest virus multiplication, if they were directly associated with this process.

Neurofibrils. As has been mentioned, Covell ('32) observed some thickening of neurofibrils in the early stages of involvement of motoneurons, but this was not observed in our protargol-stained material. Well stained and apparently normal neurofibrils were found in all cells, including severely chromatolysed ones, except those showing definite signs of necrosis.

Axons. Although the changes in peripheral nerves and nerve roots have long been recognized as resembling those of secondary or Wallerian degeneration, O'Leary, Heinbecker, and Bishop ('32) were the first to study the axonal changes in poliomyelitis in detail, from the functional as well as morphological standpoint, and to review the earlier literature. They clearly appreciated that a correlated study of functional and morphological changes was much more difficult in poliomyelitis than in the case of severed nerves, because in the latter "all axons are in approximately the same stage of alteration at the same time, while in poliomyelitis not all the cells are affected to a like degree at once". Nevertheless they established conclusively certain important facts. They showed first that motor paralysis precedes any noticeable morphological or functional changes in the axons or muscles, and that therefore the onset of paralysis is due to loss of conduction through the diseased motoneurons in the spinal cord. Moreover, the axon lesions when they appear are similar to those occurring after nerve section and occur more or less simultaneously along the length of the axon. Although they found increased irritability of fibers in affected trunks during the preparalytic and early paralytic period, they thought that the period between loss of synaptic conduction and degenerative changes in motor roots and peripheral nerves was significantly longer than that which separates nerve section and subsequent changes in peripheral stumps. This is readily explained on the basis that moto-

neurons so much damaged that they no longer conduct can still exert an adequate trophic influence on their axons.

The time course of axon changes described by O'Leary and his colleagues agrees with that which we have observed, except our material clearly shows that definite degenerative changes in ventral root fibers may occur as early as the third day of paralysis, whereas O'Leary, et al. found no changes before 4½ days. Our material consisted of reduced silver preparations of spinal cord with roots attached, permitting easy identification of roots issuing from regions with completely destroyed motoneurons. It is in such roots that one finds numerous degenerating axons on the third day of paralysis. Since some of the nerve cells giving origin to these fibers are probably destroyed on the day preceding onset of paralysis, the interval between cell destruction and visible axon deterioration is no longer than four days, and in some cases probably less. This agrees so closely with the time course of axon degeneration after nerve section (two to four days—Heinbecker, et al., '32), that the conclusion is inescapable that the degeneration of ventral root axons in poliomyelitis is a typical Wallerian degeneration. O'Leary, et al. ('32) point out that actively degenerating fibers may be found as late as 10 days, and later, following onset of paralysis, and suggest that this indicates the delayed death of cells of origin as late as 10 days after onset of paralysis. That this is not common will be shown in Part II, since very few cells show necrotic changes so late after onset of paralysis. A more probable explanation of their findings is that active degeneration of axons continues for several days after some axons have begun to deteriorate. It is interesting that Blanton ('17) found degenerative changes in the peripheral nerves of a human patient dying 36 hours after onset of paralysis. Allowing at least 24 hours for motoneuron destruction in the pre-paralytic period, one concludes that at least 2½ days could have elapsed between cell death and axon deterioration. Although Blanton thought the changes he described were too early to be due to ventral horn destruction, his findings are not contrary to the time course of typical secondary or Wallerian degeneration.

The delay in onset of axon degeneration after cell body destruction in poliomyelitis is beautifully illustrated in reduced silver preparations of the spinal cord during the first few days of the disease. In some

sections numerous motoneurons were seen, consisting only of a rounded, pale-staining mass at the site of the axon hillock, and in continuity with an axon of normal appearance. In some cases these axons were traced out into the ventral roots in the same section and were seen to be of normal appearance throughout. This picture of cell body remnant with intact axon was first illustrated, as far as could be determined, by Strauss ('10), in material from a patient who died the day after onset of paralysis.

In summary, our preparations of monkeys with abrupt onset of severe paralysis show clearly that ventral root axons of motoneurons destroyed on the first day of paralysis, and probably many on the preceding day, are morphologically intact until the third day after onset of paralysis, when degenerative changes occur all along the axon length. On the 1st and 2nd day of paralysis axons appear to degenerate centrifugally from the cell body for a short distance. It is interesting that even when inflammatory changes are most intense, including neuronophagia, no inflammatory cells of any kind are found anywhere along the axons of cells undergoing active phagocytosis. The conclusion is inescapable from this fact, and from the time course of axonal degeneration, that the primary focus of virus activity is in the nerve cell body, and that, when the cell body is destroyed, the axon undergoes typical Wallerian degeneration. Plate 4 shows the sequence of changes in anterior root fibers of limbs severely paralysed on the first day of paralysis. Figure 1 shows a normal anterior root. Figure 2 shows a degenerating root fiber in the white matter of the anterior column in an animal killed on the 3rd day after onset of paralysis. Figure 3 shows the almost universal degeneration of fibers in the anterior root of an animal killed on the 4th day. Figure 4 shows the complete absence of axons in the root of an animal killed 57 days after complete paralysis of the corresponding limb.

Nuclei. Changes in nuclei of motoneurons in acute experimental poliomyelitis have been described by Covell ('30), by Hurst ('31), and by Sabin and Ward ('41), especially with reference to the occurrence of acidophilic "inclusion bodies". Among the important findings they reported, and which our material confirms, is the fact that acidophilic inclusions, either single or multiple, are seen only in cells already showing severe cytoplasmic changes. In our experience this

holds true not only for the formation of inclusion bodies but also for the occurrence of morphological nuclear changes in general. Strauss ('10) and Blanton ('17) found the same to be true in human material. Covell found that the acidophilic inclusions gave a negative Feulgen reaction, indicating the absence of thymonucleic acid, and also reported that the inclusion bodies were most numerous soon after the onset of paralysis and diminished in numbers during the 1st to the 3rd weeks. Our findings are similar but we have observed considerable variation in the frequency of occurrence of intranuclear inclusions in different monkeys in the acute stage of the disease.

Our material indicates that nuclear changes begin after cytoplasmic changes occur, since cells showing early chromatolysis exhibit no signs of nuclear injury. Clumping of oxychromatin and the formation of discrete acidophilic inclusions occurs in cells showing fairly severe chromatolysis. In parallel with continuing cytoplasmic degenerative changes, nuclei show an apparently orderly progression of changes, as shown in Plate 1, figs. 5–8. Most commonly one notes progressive shrinkage of the nucleus and an increase in discrete chromatin masses, with progressive increase of cytoplasmic chromatolysis. When severe nuclear shrinkage is seen (Plate 1, fig. 8; Plate 2, figs. 1 and 2) the nuclear membrane is usually distorted or no longer visible, and the nucleus itself becomes intensely basophilic. The diffuse basophilia which is seen in some nuclei at this stage is usually paralleled by a similar increase of diffuse basophilia in the cytoplasm. Such cells appear to be most prone to undergo neuronophagia (Plate 2, fig. 3), and even in earlier stages seem to especially attract leucocytic and phagocytic elements. By the time phagocytosis due to granulocytes and macrophages is well under way, however, the cytoplasm has generally lost all basophilic staining (Plate 1, fig. 3).

In cells which undergo rapid lysis, or show severe vacuolation (Plate 2, figs. 6–8), the nuclei do not show the generalized basophilia noted above, and this again parallels the lack of basophilia in the cytoplasm. Such cells are not as commonly surrounded by large numbers of leucocytes and macrophages as are those showing terminal basophilia. Very often in cells undergoing lysis, the nucleus may be quite shrunken, but with basophilic granules only encircling the periphery, and with a nucleolus greatly reduced in size (Plate 2, fig. 6).

In a previous report (Bodian, '45) evidence was presented showing that the nuclei of any multinucleated motoneuron underwent parallel degenerative changes, such as shrinkage, distortion of nuclear membranes, and formation of acidophilic inclusions. The remarkable similarity in morphology of the nuclei of any multinucleated cell, in all stages of degeneration due to virus action, suggested that nuclear changes in poliomyelitis reflect the deterioration of cell functions in general rather than a direct action of virus on the nucleus.

The nuclei of persistent cells which are apparently irreversibly damaged may show various morphological appearances too diverse to suggest an orderly sequence of changes. In such cells showing evidence of retarded death and absorption the nucleus is often intensely basophilic (Plate 5, figs. 2–4), may be swollen or shrunken, or may appear to be relatively intact, as if resisting degeneration more than the severely injured cytoplasm (Plate 5, figs. 1 and 5). In some of these cells the nucleus appears to be on the verge of being extruded, although relatively normal in appearance (Plate 5, fig. 6; Plate 10, fig. 1). The cytoplasm in persistent severely damaged cells is generally highly eosinophilic or hyalin in appearance. At times such cells exhibit a ring of peripheral Nissl substance of abnormal appearance, quite unlike that seen in most cells in the stage of central chromatolysis.

In the subacute stage and later recovery periods, the nuclei of cells with normal cytoplasmic structure are generally normal in appearance as well. Occasionally, however, large persistent acidophilic inclusion bodies are seen in cells of otherwise quite normal appearance (Plate 9, fig. 4). Since in the acute stage such inclusions are seen only in severely chromatolysed cells, their presence later in cells otherwise normal is evidence of the power of recovery of cells damaged by virus action. Such inclusions have been seen in otherwise normal cells as late as 49 days after onset of paralysis. Rarely one sees in otherwise normal cells an unusual nuclear "inclusion" consisting of the nucleolus surrounded by a closely packed aggregate of basophilic material (Plate 9, figs. 5 and 6). This appearance is also occasionally seen in severely damaged cells (Plate 10, fig. 2). This bizarre intranuclear formation in otherwise normal cells has not been seen in formative stages, so that no interpretation of their development seems feasible at present.

Criteria of Necrobiosis in Motoneurons. It is evident from the pre-

ceding paragraphs that certain extremé cytopathological changes were associated with the processes of necrobiosis or irreversibility of injury following poliomyelitic infections. Among the irreversibly injured cells are, of course, those destroyed and completely absorbed, and those which persist for variable periods of time before being absorbed, and designated hereafter as "necrotic". The process of cell destruction and complete absorption in poliomyelitis must occur at times in the space of a few hours, since in our earliest preparalytic cases one already finds cells in this category whose only mark is either a vacant space in the section ("falling out"), or a cluster of phagocytic cells filling in the spaces formerly occupied by the neurons. Many nerve cells, however, die more slowly, presumably after passing through the usual stages of chromatolysis. Unlike the early stages of degeneration no single orderly sequence of morphological stages characterizes the processes of deterioration from the time of cell "death" to the time of complete disappearance of the cell. Cells which we have designated as necrotic may show severe chromophilia or complete loss of stainability ("ghost cells"), vacuolation and lysis without neuronophagia or signs of neuronophagia without lysis, severe nuclear change including pyknosis or extrusion of a relatively intact nucleus. The disorderly variation of morphological appearance after complete loss of Nissl substance suggests first that such complete loss is incompatible with recovery of the cell, and second, that subsequent cellular processes are quite out of control and may lead to several alternative morphological sequences leading to complete cell destruction. It is interesting that Horanyi-Hechst ('35) has described quite similar terminal stages of motoneuron degeneration in human cases.

In *summary*, the following cytological changes appear to be associated with irreversible neuron injury, and have been considered as indicators of necrosis:

1. Complete loss of cytoplasmic basophilia. 2. Disappearance of mitochondria. 3. Breakdown of neurofibrils. 4. Severe nuclear changes, other than the formation of "inclusions". This includes severe nuclear pyknosis, complete loss of nuclear basophilia, rupture of nuclear membrane, or nuclear extrusion. 5. Cytoplasmic vacuolation. 6. Glassy appearance of cytoplasm, with some Nissl substance still remaining in the periphery. 7. Severe cellular basophilia, usually associated with beginning neuronophagia. 8. Neuronophagia.

II. The Sequence of Pathological Changes in the Anterior Horn, with a Statistical Study of Injury and Recovery Stages in Motor Nerve Cell Populations

It is possible to examine individual nerve cells in poliomyelitis in various stages and to draw certain inferences regarding the sequence of events in their injury and recovery stages. Heretofore, this has been done largely with respect to the injury stages by workers like Covell ('32), Hurst ('29), and Sabin and Ward ('41). Many of such inferences, although reasonable, are impossible to prove without quantitative data regarding the cell population as a whole, and moreover cannot be related to the entire disease picture without a statistical approach. Such an approach should be capable of revealing important data impossible to obtain by the study of individual cells. The pathogenesis of paralysis, for example, bears no relation to the fate of any individual cells, since the cells in the nerve cell population supplying the arm of a rhesus monkey number about 14,000 and at any particular time in the acute stage may be in widely varying stages of pathological change. The paralysis can only be related to the status of the cell population supplying the muscle or groups of muscles under consideration. Similarly, at the present time, the concentration of virus in infected tissue can be related only to a cell population in the tissue being studied.

It is the purpose of the following pages to present first a survey of the principal changes occurring both in the motoneuron population and in the populations of reacting mesodermal glial cells during sequential clinical stages of the disease. With this survey in mind, the statistical analysis of changes in the motoneuron population will then be more readily understood.

Pathological Characteristics of Successive Clinical Stages

The preparalytic period

The most informative stage in the pathological process is the earliest in which visible changes are apparent. This stage precedes paralysis by about 1 to 3 days, and gives important information about the sequence of early changes in neurons as well as in the inflammatory response. The important findings may be summarized as follows, before we proceed to the details:

1. The most common visible change in nerve cells in the earliest stage is a diffuse thinning of Nissl bodies throughout the cell body, often most conspicuous around the nucleus. Various degrees of this may be seen, but it is only in the late preparalytic stage that complete dissolution of Nissl bodies is found in large numbers of cells.

2. Nuclear changes are, as a rule, not apparent until severe cytoplasmic chromatolysis has occurred. The earliest nuclear change is usually the formation of acidophilic inclusion bodies.

3. Changes in mitochondria are not apparent in cells showing mild or even severe chromatolysis (also McCann, '18; Hurst, '29) but very few mitochondria remain when presumably irreversible changes have occurred in the cell ("acidophilic necrosis", nuclear dissolution or pyknosis, cell vacuolation, neuronophagia).

4. Neurofibrillar changes are not apparent until severe cell changes have occurred (also Hurst, '29).

5. The entire sequence of neuron changes from early injury to cell necrosis is present in the earliest pathological focus in our series, although frankly necrotic cells are few in number. Since recovery changes in nerve cells at this time are hardly to be expected, all changes may be assumed to be progressive and have been arranged in sequence, according to severity, in Plate 1 (figs. 1–8).

6. Changes in nerve cells of mild or severe degree in the preparalytic period are entirely independent of inflammatory changes, as shown by Hurst ('29), and may occur in regions of ventral horn gray matter where no inflammatory response has occurred. Nevertheless, leucocytes may be present in considerable numbers in other regions where nerve cell changes are so slight that they are not clearly outside of the range of normal.

Monkeys 959 and A935 were most instructive in revealing the earliest inflammatory and neuronal changes in our series. Rhesus 959 was inoculated intracerebrally with MV virus 5 days before he was killed, at which time the only symptom was that of fever, present for 24 hours. No lesions were present in serial sections of the lumbar cord. The cervical cord contained only two pathological foci, one restricted to about 100 15μ sections within the 6th cervical segment, and the other restricted to 32 sections within the 7th cervical segment. The focus in C6 contained no interstitial infiltration, but only very light

perivascular infiltration in the dorsal parts of the anterior horns and in the intermediate gray horns, especially around the branches of vessels in the ventral fissure. The infiltrating cells were largely granulocytes, with a sprinkling of mononuclear cells. Within this region of the cord no definite changes in nerve cells were visible except chromatolytic changes which were so slight that they could have been within the range of normal.

The focus in C7 was more advanced than that in C6 and was remarkably restricted to the left anterior and intermediate gray horns, both with respect to neuronal and to inflammatory changes. The only vessel outside of this territory showing perivascular infiltration was the artery of the ventral fissure, and only the left branches of this vessel were "cuffed". Within this focus the perivascular infiltrations contained more mononuclear cells and relatively fewer granulocytes than in the earlier focus in C6. Perivascular leucocytes were present around vessels of all sizes from capilaries to the vessels of the ventral fissure. At the periphery of this focus there were still no diffuse or focal extravascular infiltrations, although some neurons showed diffuse cytoplasmic chromatolysis of a definite degree. These neurons showed no nuclear changes as yet and no leucocytes or excess of glia cells were to be seen near them. In the center of the focus, granulocytes and some lymphocytes were seen in the parenchyma in small numbers as well as in perivascular spaces. Granulocytes could be seen surrounding neurons of normal appearance, or around cells in any stage of chromatolysis or degeneration. They were numerous in the vacuoles or "bays" of hyperchromatic necrotic neurons, but were rarely seen near "ghost" neurons or severely autolysed ones. It is important to note that lightly or severely damaged neurons may or may not be surrounded by infiltrative cells. One may therefore conclude that, although breakdown products associated with the earliest neuronal changes can attract migrating leucocytes from the vessels, not all damaged neurons exert this influence.

The cytological changes in the neurons of this focus were of great interest. Within the 32 15μ sections in the segment of cord which contained the focus it was found by actual count of all motoneurons that there were a total of 442 in the lateral column of the right anterior horn, and 451 in the corresponding region on the left side. All neurons on the

right side were normal in appearance and no infiltrative changes were present. On the left side 52% of the neurons were normal in appearance, 28% showed mild degrees of diffuse cytoplasmic chromatolysis, without nuclear changes, 16% showed severe cytoplasmic chromatolysis, and such cells often contained nuclear inclusion bodies, and 4% of the neurons showed necrotic changes. Only one cell was in the process of neuronophagia. In all other preparalytic cases, a similar distribution of pathological cell types was found, with however more cells actually destroyed or necrotic (Table 3). Thus, on the basis of frequency of occurrence, one can safely reconstruct the sequence of neuronal changes from the earliest visible ones to the stages of cell breakdown, as shown in Plates I and II.

It is interesting that in the preparalytic period, among six animals examined in detail, it was exceedingly rare to find cells showing central chromatolysis, although this pattern of Nissl body distribution is quite common in the subacute stage, as will be seen. Moreover, in no regressive stage in the preparalytic period does one find the nuclear eccentricity characteristic of the chromatolysis which follows axon amputation.

In later stages of the preparalytic period, polyblasts may be present in large numbers as well as the numerous leucocytes. Granulocytes appear to be more numerous in the lumbar than in the cervical cord as a rule, as also noted by Hurst ('29), by Pette, et al. ('32) and by Covell ('32). This was also true in Blanton's earliest human case of 36 hours' duration ('17). In some individuals they may be present in enormous numbers in the gray matter, and in such cases appear to migrate into the surrounding white matter in large numbers.

Another preparalytic case (A935) is especially instructive in revealing very early pathological changes, and in emphasizing two important aspects of pathogenesis. In this case, which at some levels of the cord shows characteristic and fully-developed neuronal and inflammatory lesions (Plate 6, figs. 1 and 2), one finds at adjacent levels sections which contain only a single motoneuron of abnormal character (Plate 6, figs. 3, 4 and 5). Such isolated neurons showed the severe diffuse chromatolysis typical of early poliomyelitis and occurred in several sections. Of considerable interest is the fact that no infiltrative cells were present in the anterior horn gray in the region surrounding such

neurons, although early perivascular infiltration was present surrounding the main branches of the anterior spinal vessels (Plate 6, fig. 2). Such findings strikingly confirm Blanton's ('17) in human cases and Hurst's ('29) in monkeys, in demonstrating that neuronal chromatolysis, even of severe degree, may occur prior to and not as a result of inflammatory changes. It is interesting that Pette, Demme and Környey ('32) also found early neuron changes in the absence of cellular infiltration, but were not sure that such changes were not artifacts of histological preparation. In our material, the isolated chromatolytic cells were found in any position of the anterior horn cross-section, whether dorsal, ventral, medial, or lateral. There was no suggestion of a progression of lesions from the dorsomedial position of the horn lateralwards, as postulated by Elliott ('45). At least in the early stage of the disease, the spread of virus in the gray matter does not occur by cell-body to cell-body extension, but probably along axon connections in which cytopathological changes cannot be observed. The question is raised, in this connection, whether the often postulated spread of virus liberated from destroyed cell-bodies to adjacent cell-bodies occurs at all, since, as will be shown below, extensive dissemination of neuron lesions may occur before widespread nerve cell destruction. Moreover, the highest virus titers may be achieved in the preparalytic period before extensive cell destruction occurs, and presumably as a result of virus multiplication in many cells (Bodian and Cumberland, '47). In cases with little paralysis, inoculated with "mild" strains, extensive motoneuron chromatolysis occurs and high titers are obtainable, with only a small proportion of destroyed nerve cells.

The first day of paralysis

In animals in which the onset of paralysis is abrupt, histopathological changes in the spinal cord resemble closely those seen on the day preceding expected paralysis, but are generally more widespread and more severe. Although the predominant cytopathological stage in motoneurons is that of diffuse chromatolysis, numerous cells in various stages of necrosis and neuronophagia can usually be seen. Such cells are most common at this time as compared with preceding or following days. Advanced stages of chromatolysis of Nissl substance, including

complete disappearance of the latter, predominate, with some cells showing cytoplasmic vacuolation or autolysis. Nuclear changes are rarely severe except in cells showing severe chromatolysis, and in such cells intranuclear acidophilic inclusions are common. Acidophilic inclusions generally occur in cells with otherwise relatively normal nuclei whereas basophilic intranuclear "inclusions" are found at times in cells which have either shrunken or highly vesicular nuclei.

Neurons about to undergo neuronophagia are common at this stage, and are generally highly basophilic. In later stages of active neuronophagia neurons are strongly eosinophilic, suggesting perhaps that the neurophages produce enzymes, possibly nucleases, which remove the basophilia. Many neurons are destroyed, become autolysed, and are absorbed without any evidence of participation by neurophages, and both removal by autolysis and by neuronophagia may be so rapid that even on the first day of paralysis the location of many motoneurons may be marked only by a vacant space ("falling out"), or by a compact cluster of neurophages.

The inflammatory process on the first day of paralysis is in general more severe than on the preceding day, with a tendency, however, for reduction in numbers of granulocytes. The presence of much cellular debris, and apparently nuclear fragments, in some foci may mark the site of heavy infiltration with such cells, and suggests that their rapid disappearance is due to destruction *in situ*. During the period of early and increasing paralysis the lymphocytic cells are predominant in numbers, although macrophages are also quite numerous.

Second and third days

The outstanding feature of the histopathological picture at this period is the scarcity of normal-appearing neurons, even in cases with mild paralysis. Not a single case examined at this period showed more normal cells than 31% of the expected number. This indicates the wide dissemination of virus in the spinal cord of paralytic cases, and also supports evidence from other sources, to be considered later, that many cells are invaded by virus and later recover. Residual neurons are predominantly in late stages of diffuse chromatolysis, although, for the first time, cells showing central chromatolysis may make their appearance in appreciable numbers.

At this period most of the destroyed neurons are completely absorbed, with only focal accumulations of neurophages to mark the spot. Early stages of neuronophagia are also seen, but are not usually as numerous as on the first day of paralysis. In regions of extensive cell loss, even the foci of neuronophagia may have cleared up, so rapid is the sequence of changes, and large areas of anterior horn, devoid of nerve cells, may be characterized by a diffuse increase of mononuclear cells.

The principal cells in the inflammatory response, at this time and in most cases, are the polyblasts of Maximow, as shown by Wickman ('13). These cells, with rod-shaped or irregular nuclei, have also been identified as microglia by various observers. Other cells of the lymphocytic series may also be very numerous, especially in the perivascular accumulations of cells. Normal-appearing granulocytes are uncommon, and their disappearance as such is marked by the occurrence of much basophilic nuclear debris which probably represents their remains.

Silver preparations at this stage show the earliest phases of axon degeneration in ventral root fibers, occurring only in cases killed at least 2½ days following the onset of paralysis. These changes have been described in the first section of this report, and require no further comment.

Four to six days

This period is remarkable from the morphological as well as from the functional point of view. The cases, like those in the preceding period, show a very low percentage of normal neurons, even in cases with surprisingly little functional loss, and with cessation of increase of paralysis. Since very little, if any, active neuronophagia is in evidence, and since necrotic stages of any sort are rare, it is likely that surviving cells would have recovered even though many are severely chromatolytic.

This period is also interesting in showing a reversal of the striking tendency of damaged neurons in the preceding periods to show diffuse chromatolysis in the cytoplasm. Now many cells show aggregation of Nissl substance near the cell and nuclear membranes. Cells showing this type of "central chromatolysis" (Plate 7, figs. 4–6) are mixed with those showing the diffuse type (Plate 7, figs. 1 and 2), and many

transitional stages are also seen (Plate 7, fig. 3). Occasionally in the same individual one finds cells with diffuse chromatolysis predominating in one spinal cord segment and those with central chromatolysis predominating in a neighboring segment. Eccentricity of nuclear position appears in some cells showing "central" chromatolysis.

The axons of motoneurons reveal interesting changes at this stage. In segments of spinal cord in which extensive destruction of motoneurons has occurred, many axons in the ventral roots show severe degenerative changes, and, in some cases, no intact axons are visible (Plate 4, fig. 3). In segments where many neurons remain, most axons in the roots are normal in appearance, *even when most neurons show severe chromatolytic changes*. This finding confirms other suggestions, previously mentioned, that axon degeneration is a consequence only of actual cell body destruction.

Seven to twelve days

The principal feature of neuron morphology in this period is the predominance of "central" chromatolysis in remaining cells other than normal-appearing ones (Plate 8, figs. 1–5). Cells showing diffuse chromatolysis or necrotic changes, as previously defined, are few in numbers as a rule. The one exception to this rule was found in case B23, in which many cells in severe diffuse chromatolysis were found. This suggests a possible association with continuing virus activity, since in exceptional cases this may be found as late as the third week after onset of the disease (Brodie, '33; Bodian and Cumberland, '47).

Several features of the pathological picture in this period give evidence that the recovery process is well under way. A greater proportion of normal or almost normal neurons is found than in the preceding period. In some cases numerous cells are seen with normal or almost normal Nissl body pattern, in which, however, large inclusion bodies are found in the nucleus, indicating previous cytoplasmic changes of a fairly severe degree (Plate 8, fig. 6, b). Although the inclusions are usually acidophilic, many at this stage in cells of otherwise normal appearance are larger than usual and seem to consist of an aggregate of basophilic particles which surrounds the nucleolus, converting it into a core for this bizarre formation. Such nuclear "inclusions" are generally surrounded by a shrinkage space, and are observed as late as

35 days after onset of paralysis (Plate 9, figs. 5 and 6, a). Although the cytoplasm in such cells is generally normal or almost so, it is problematical whether cells with such marked nuclear change can continue to recover, since in a few cases cells with nuclear inclusions of this type were seen in a condition of obvious necrosis (Plate 10, fig. 2). Finally, in this period there is a tendency for disappearance of lymphocytes which were previously scattered throughout the parenchyma in company with polyblasts or macrophages. At the same time the number of lymphocytes in the perivascular infiltrations increases considerably, so that "cuffing" is more prominent than before. This increase is in part due to formation of lymphocytes *in situ*, as evidenced by numerous mitotic figures, but may also be aided by accretion from lymphocytes free in the tissue. The disappearance of the lymphocytes free in the tissue, however, is probably more often due to transformation into polyblasts, as described by Maximow ('27). Some lymphocytes appear to undergo phagocytosis by macrophages, but this is not often observed (Plate 10, fig. 2, m). At any rate, during the second week the population of "replacement" cells in the parenchyma is predominantly polyblasts, and variations of these such as "gitter" cells and "rod" cells are numerous. Although the polymorphonuclear leucocytes have a short period of prevalence as infiltrating elements, namely the first day or two of the disease, and cells of the lymphocyte group a somewhat longer period, the first week of the disease, the histiocytic cells or macrophages are the most prevalent after the first week and predominate among the non-neuronal cellular elements for several months.

The third week

During the third week after onset of paralysis, less than 10% of all neurons are still abnormal in appearance, so rapid is the recovery process. Most of the nerve cells, by this time, are either normal in appearance or destroyed and removed without remaining traces. A few irreversibly damaged cells of the type shown in Plate 5, figs. 1–6, may be seen in some cases as late as this period, but such persistent "necrotic" cells are negligible in numbers as compared with those quickly destroyed and removed during the first few days of the disease. The predominant pathological stage is that of "central" chromatolysis, with evidence of recovery of Nissl substance (Plate 9, figs. 1 and 2). Intranuclear inclusions may be present (Plate 9, fig. 2, b).

The increased size of the perivascular infiltrations, noted in the previous period, is even more striking in some cases during the third week, when some of the larger "cuffed" vessels attain massive proportions. Around other vessels, especially the smaller ones, the lymphocytes tend to disappear, and are replaced by spindle-shaped histiocytes. This process is seen as early as one week after onset of paralysis (Plate 10, fig. 2), but is more common during later periods. It is interesting that occasionally one finds on the same vessel a locus of heavy lymphocytic layering, adjoining a site where histiocytes greatly predominate in the thickened adventitial layer. In the parenchyma itself in cases during the third week, numerous histiocytes are found, with a small sprinkling of remaining lymphocytes.

Thirty-five to forty-nine days

The cytopathological picture in the cases in this period resembles that in the preceding one. However, no neurons with severely abnormal appearance and which might be considered as moribund were found. A very small proportion of chromatolytic neurons were observed (Plate 9, figs. 3 to 6), and other remaining neurons were essentially normal in appearance. Large acidophilic nuclear inclusion bodies were found in a few cells of otherwise normal appearance (Plate 9, fig. 4, b), as late as 49 days after onset.

The persisting lymphocytic perivascular infiltrations in some instances assume the proportions of small lymphatic nodules in this period (Plate 11, figs. 1 and 2). Other regions of the cord in the same individual, however, may be nearly free of lymphocytes at this time and exhibit histiocytes as infiltrating cells almost exclusively. In some cases the heavily "cuffed" vessels show basophilic stem cells in the center of the mass of lymphocytes, and mitotic figures which give clear evidence of active lymphocytopoiesis (Plate 11, fig. 1).

Convalescent stage

The morphological recovery of over 90% of surviving neurons is attained at the end of the first month of the disease. During the next few weeks, as indicated above, the residual chromatolytic cells are negligible in number, and moreover the number of lymphocytes free in the tissues is reduced to very small proportions. It is interesting that the essential disappearance of leucocytic infiltration, which

marks the close of the active inflammatory process, should be roughly coincident with the recovery of most remaining neurons. Both processes take place during the subacute period, following which the further stages of recovery appear to take place at a slower rate. Occasional chromatolytic cells may still be found several months after onset of the disease, for example. Similarly, heavily cuffed vessels may persist for at least a year, although such cuffing is gradually reduced markedly after the first 6 months of the disease. During the late convalescent period histiocytes also are greatly reduced, and affected regions of anterior horn are characterized by a variable degree of gliosis, which marks the terminal stage of the non-neuronal reparative process.

Summary of Quantitative Data

Dynamic Aspects of the Infection and Recovery of the Motoneuron Population

When changes in motoneurons during a series of stages in the acute period are considered in a qualitative way, the variety of morphological appearances is such as to render difficult a clear appreciation of the significant phases of a continuous process. In their very thorough study of histopathological changes in the acute period, for example, Pette, Demme, and Környey ('32) were more impressed with the variety of neuron changes than with a possible orderly progression of changes. This problem is easily dissipated when the disease in the spinal cord is considered as an "epidemic" occurrence in the nerve cell population. With this point of view in mind, the *prevalence* of each stage in the regression and recovery of nerve cells can be treated in a quantitative way throughout the course of the disease. Since in each animal the motoneuron population is studied at a single point of time in the disease, and since considerable variability in the severity of the disease occurs from animal to animal, it was decided to select at each principal stage a group of about five animals with approximately the same average severity of paralysis. This represents a group of 20 limbs, supplied by motoneurons of which a total sample of about 10,000 was considered in the quantitative study. The prevalence of each morphological stage was tabulated for the motoneuron population supplying each extremity, and the average prevalence for all extremities in

all animals of a given group then obtained (Tables 3 to 10; Charts 2 and 3). The averages allow a clear understanding of the time course of pathological changes, as shown in the charts, whereas any given animal may represent an extreme case of variation from the normal which may be difficult to assess in relation to the common sequence of events.

For the purposes of an epidemiological study it is fortunate that the nerve cell population in an infected animal is absolutely stable with respect to factors such as migration and multiplication. The number of cells can be presumed to be constant in number and location, except for decrease by deaths due to the disease, since mature neurons do not multiply or migrate. It is necessary only to define the precise cell population involved, and to treat the frequency of occurrence of each stage in the regression and recovery of nerve cells in a quantitative way throughout the course of the disease.

Determination of the distribution of the normal motoneuron population. In order to assess the lamage to motoneurons due to poliomyelitic infection it was necessary to identify the motoneuron population in normal animals and to establish the pattern of distribution of the neurons which principally innervate each limb. Since there is some admixture of such neurons with non-motor internuncial neurons in the anterior horns, the motoneurons were identified by means of the method of retrograde chromatolysis. In four monkeys the entire left brachial plexus was sectioned in the subclavian region, and the left lumbosacral roots were sectioned in the intervertebral canals. In three additional monkeys, the brachial plexus alone was sectioned. After periods of from 7 to 30 days, during which time the cells of origin show clear-cut chromatolysis, these animals were prepared and examined in a manner similar to that described above.

After interruption of the limb plexuses it was found that only about 1% of the chromatolytic cells were in positions outside the lateral and central cell columns of the anterior horns. The apparently displaced chromatolytic cells were entirely in the medial cell columns and were probably the result of operative injury to the back muscles. Since the area of sections containing the chromatolytic cells was therefore essentially the area containing the lateral and central cell columns, it could readily be identified in normal or pathological material. The limits of

this area dorsally and dorsomedially can be defined for all practical purposes as the limits of the very large cells (cell bodies over 50μ). In the lumbar region this area is particularly clearly defined, as shown in Plate 12.

The chromatolytic preparations also served to differentiate the motoneuron population from internuncial cells in the same area and to show that the latter introduced but a small error in studying poliomyelitis preparations, providing that only cells over 25μ in diameter were considered. It was found, for example, that 80 to 95% of cells over 25μ in diameter showed chromatolysis in the lateral and central cell columns after root or plexus section. Only about 30% of cells smaller than 25μ showed chromatolysis, and this is probably a high figure since some small cells show apparent chromatolysis in quite normal animals. Since such small cells represent only about 20% of the total cell population in the region considered, an error in estimating motor cells of at most 6% is introduced by neglecting small cells. These relationships are shown graphically in Chart 1.

Chart 1 also shows the variation in both normal and chromatolytic cells in various segments of the spinal cord enlargements. As can be seen by examining the curve of mean values, there is a definite trend towards greater concentration of large motoneurons in the two cervical segments C7 and C8, and in the two lumbar segments, L6 and L7. Consequently an average figure for the number of motoneurons on one side in a single 15 micra section was derived for each segment from counts of normal preparations. This is shown in Chart 1 for large and small neurons separately, and the total number of cells per section shown in comparison with the number showing chromatolysis after plexus section.

In order to determine the number of motoneurons destroyed in poliomyelitic cases, the number obtained by counting the large cells (over 25μ) in a sample of 10 to 15 sections per spinal segment was subtracted from the number which one would expect to find if the cord were normal. The "expected number" was obtained by multiplying the number of sections examined in each segment by the average number of large cells per side per section previously determined for the segment in question. The sum of the totals of individual segments was then the "expected number" of large cells which should have been present on one side in the sections taken as a sample of the cord enlargement.

The "expected number" is thus close to being the average number of motoneurons present in a sample of 40 to 60 sections through a limb enlargement, and supplying a single limb. It is subject to the errors of including about 10% of large cells which may not be motoneurons, of

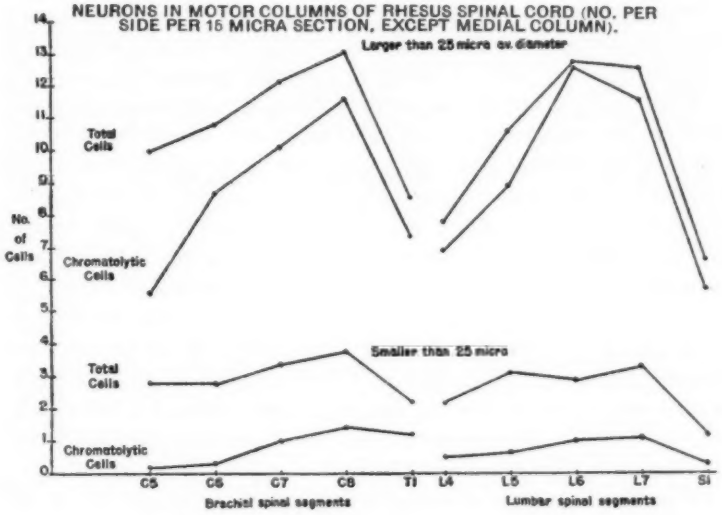
NEURONS IN MOTOR COLUMNS OF RHESUS SPINAL CORD (NO. PER SIDE PER 15 MICRA SECTION, EXCEPT MEDIAL COLUMN).
Larger than 25 micra av. diameter
Total Cells
Chromatolytic Cells
No. of Cells
Smaller than 25 micra
Total Cells
Chromatolytic Cells
C5 C6 C7 C8 T1 L4 L5 L6 L7 S1
Brachial spinal segments
Lumbar spinal segments

CHART 1. FREQUENCY DISTRIBUTION OF ANTERIOR HORN CELLS IN LIMB SEGMENTS OF RHESUS SPINAL CORD.

Cell counts were made in area of cord containing neurons over 50μ in diameter, and showing chromatolytic cells after plexus or root section. Chromatolytic cells in brachial region are the consequence of brachial plexus section, whereas those in lumbar segments are due to section of third to seventh lumbar roots. Each point on the curves represents the combined means of values from 7 or 8 monkeys, except for the small cells, which are obtained from 2 monkeys. In each monkey the average value for cells per section in each spinal segment was obtained by counting the cells in a sample consisting of 10 to 15 sections. Only cells containing nucleoli were counted.

excluding about 6% of small cells which may be motoneurons, and of an individual variation from case to case of about 10%, when the whole region is considered. Errors of this magnitude are not serious in comparing neuron destruction with loss of motor function, since the clinical

estimate of muscle power of a limb is less accurate than the estimate of the number of cells in the corresponding motoneuron population.

As a further and final check on the accuracy of determination of the normal control motoneuron population, a comparison was made between the total "motoneuron" count on one side of the brachial (C6 to T1) and lumbar enlargements (L4 to L7) and the counts of nerve fibers in corresponding ventral roots in rhesus monkeys made by Larsson ('38). In reduced silver preparations Larsson found a total of 15,795 fibers in the ventral roots of C6 to T1 inclusive, and 16,347 fibers in the ventral roots of L4 to L7 inclusive. On the basis of our counts of all chromatolytic cells, large and small, in the lateral and central cell columns of corresponding cord segments in our material, an estimated number of 12,000 for the brachial and of 12,600 for the lumbar enlargements was obtained. The latter figures, of course, did not include cells in the medial column which contribute axons to the ventral roots, and which therefore are represented in Larsson's figures. Although Larsson's counts were based on a single rhesus monkey, the agreement between our figures, after discounting the discrepancy based on our omission of cells in the medial column, suggests a reasonably good correspondence between ventral root fibers and cells which we have defined as "motoneurons".

Changing proportions of normal, abnormal, and destroyed cells in the total motoneuron population. A general survey of the course of the disease in the motoneuron population can be obtained by an analysis of the changing proportions of three principal groups of cells in the total population, the normal cells, the abnormal ones, and those totally destroyed. The total population is estimated in the manner previously described and called the *expected number* of nerve cells. The destroyed cells are estimated by subtracting the combined groups of surviving normal and abnormal cells from the expected number.

In Tables 3 to 10 these data are given for each case in the columns headed "Per Cent of Expected Number", and the mean values for all cases in each group are plotted in Chart 2. Although, as might be expected, the variation in severity of maximal paralysis is associated with variation in proportion of cell groups in individual cases from the same period, the mean values clearly reveal the trend of events in the populations. The same trends are reflected to a lesser degree in the maximum and minimum values for each, shown in Tables 3 to 10.

TABLE 3

Preparalytic Period

Analysis of types of neuron damage in samples of motoneuron population supplying arm or leg

ANIMAL AND EXTREMITY	MAXIMAL PARALYSIS	TOTAL CELLS COUNTED	EXPECTED NO. IN SAMPLE	ESTIMATED DESTROYED PLUS ABNORMAL	% OF EXPECTED NO.			% OF REMAINING CELLS					
								Diffuse Chromatolysis (Regressive)		Central Chromatolysis (Recovery)			
					Normal	Abnormal	Estimated Destroyed	Mild	Severe	Mild	Severe	Necrotic	Normal
959													
R. Arm	5	422†	442	0	100	0	0	0	0	0	0	0	100
L. Arm	5	451‡	451	216	52	48	0	28.0	15.8 (4.9)*	0	0	4.2	52.0
B474													
R. Arm	5	423	502	188	62	22	16	9.7	12.3 (5.0)	0	0	3.8	74.2
L. Arm	5	453	502	102	80	10	10	7.5	2.2 (1.1)	0	0	2.0	88.3
B8													
R. Arm	5	454	537	165	69	15	16	7.0	6.6 (1.5)	0	0	4.4	82.0
L. Arm	5	423	537	187	65	14	21	6.4	5.4 (1.4)	0	0	5.4	82.8
R. Leg	5	483	526	107	80	12	8	7.3	5.0 (2.3)	0	0	1.0	86.7
L. Leg	5	482	526	79	85	7	8	5.6	0.6 (0)	0	0	1.0	92.8
B12													
R. Arm	5	449	591	338	43	33	24	8.2	15.8 (8.9)	0	0	19.6	56.4
L. Arm	5	437	591	337	43	31	26	5.7	13.5 (8.9)	0	0	22.7	58.1
R. Leg	5	551	627	250	60	28	12	10.9	8.8 (5.4)	0	0	11.8	68.5
L. Leg	5	556	627	148	77	12	11	7.1	4.5 (2.7)	0	0	2.3	86.1
B22													
R. Arm	5	464	552	325	41	43	16	26.7	17.9 (6.5)	0	0	6.5	48.9
L. Arm	5	324	552	519	6	53	41	35.2	37.3 (10.7)	0	0	17.3	10.2
R. Leg	5	423	475	353	26	63	11	36.4	22.7 (9.5)	0	0	12.1	28.8
L. Leg	5	448	475	303	36	58	6	34.8	18.1 (5.4)	0	0	8.7	38.4
Mean.....		455	538	241	55	30	15	15.8	12.4 (4.9)	0	0	8.2	63.6

† This side not included in calculations because entirely normal.

‡ Complete count of single focus in 7th cervical segment.

* Figures in parentheses in this and subsequent tables represent percentages of severely chromatolytic cells which are completely chromatolysed or almost so.

As was previously shown (Bodian and Cumberland, '47), the pathological process very commonly has its onset in rhesus monkeys on the

TABLE 4

Early Acute Period (1st day)

Analysis of types of neuron damage in samples of motoneuron population supplying arm or leg

ANIMAL AND EXTREMITY	MAXIMAL PARALYSIS	TOTAL CELLS COUNTED	EXPECTED NO. IN SAMPLE	ESTIMATED DESTROYED PLUS ABNORMAL	% OF EXPECTED NO.			% OF REMAINING CELLS					
								Diffuse Chromatolysis (Regressive)		Central Chromatolysis (Recovery)			
					Normal	Abnormal	Estimated Destroyed	Mild	Severe	Mild	Severe	Necrotic	Normal
A696													
R. Arm	5	364	444	153	66	16	18	9.1	7.1 (1.6)	0	0	3.8	80.0
L. Arm	5	436	444	85	81	17	2	12.1	3.2 (0.5)	0	0	2.3	82.4
R. Leg	5	377	483	412	15	63	22	39.6	32.8 (9.0)	0	0	8.8	18.8
L. Leg	4	313	483	416	14	51	35	32.9	33.6 (12.5)	0	0	12.1	21.4
C725													
R. Arm	2	288	484	478	1	58	41	9.7	42.3 (38.2)	0	0	45.9	2.1
L. Arm	2	408	484	446	8	76	16	13.0	63.8 (54.7)	0	0	13.9	9.3
B26													
R. Arm	4	350	514	423	18	50	32	26.6	30.3 (12.9)	0	0	17.1	26.0
L. Arm	5	413	514	435	15	65	20	28.4	31.9 (12.3)	0	0	20.6	19.1
R. Leg	5	425	499	309	38	47	15	41.0	10.1 (5.2)	0	0	4.2	44.7
L. Leg	5	418	499	323	35	49	16	36.4	12.4 (4.3)	0	0	9.1	42.1
B11													
R. Arm	0	47	512	512	0	9	91	4.3	76.6 (53.2)	0	0	19.1	0.0
L. Arm	3	218	512	508	1	42	57	23.4	47.2 (20.6)	0	0	27.5	1.9
R. Leg	5	425	563	370	34	41	25	40.4	8.7 (3.1)	4.0	0	1.4	45.5
L. Leg	5	423	563	512	9	66	25	19.2	32.9 (21.5)	0.2	0	35.7	12.0
B29													
R. Arm	3	362	543	506	7	60	33	30.1	26.5 (14.4)	0	0	33.2	10.2
L. Arm	5	416	543	364	33	44	23	38.7	13.0 (4.6)	0	0	5.3	43.0
R. Leg	5	385	515	283	45	30	25	27.0	6.5 (3.1)	0	0	6.2	60.3
L. Leg	5	462	515	182	65	25	10	27.5	0.4 (0)	0	0	0.0	72.1
Mean.....		363	506	373	27	45	28	25.5	26.6 (15.1)	0.2	0	14.8	32.8

day preceding onset of paralysis. At this time in the preparalytic period there is already apparent a sharp decline in the proportion of normal cells. This decline continues at about the same rate until the

TABLE 5

Acute Period (2–3 days)

Analysis of types of neuron damage in samples of motoneuron population supplying arm or leg

ANIMAL AND EXTREMITY	MAXIMAL PARALYSIS	TOTAL CELLS COUNTED	EXPECTED NO. IN SAMPLE	ESTIMATED DESTROYED PLUS ABNORMAL	% OF EXPECTED NO.			% OF REMAINING CELLS					
								Diffuse Chromatolysis (Regressive)		Central Chromatolysis (Recovery)			
					Normal	Abnormal	Estimated Destroyed	Mild	Severe	Mild	Severe	Necrotic	Normal
A816 (2 d.)													
R. Arm	1	44	402	402	0	11	89	9.1	77.2 (38.6)	0	0	13.7	0
L. Arm	1	127	402	401	0	31	69	14.2	72.4 (40.9)	0	0	12.6	0.8
C924 (2 d.)													
R. Arm	3	462	558	483	13	70	17	25.8	57.6 (24.0)	0	0	0.4	16.2
L. Arm	3	474	558	411	26	59	15	23.6	45.3 (13.9)	0	0	0	31.1
R. Leg	1	190	658	658	0	29	71	2.1	83.7 (25.3)	0	0	14.2	0
L. Leg	1	139	658	658	0	21	79	3.6	77.7 (20.1)	0	0	18.7	0
C817 (2 d.)													
R. Arm	0	50	532	532	0	9	91	12.0	78.0 (32.0)	0	0	10.0	0
L. Arm	2	278	532	526	1	52	48	29.8	62.2 (12.2)	0	0	5.8	2.2
R. Leg	2	226	568	568	0	40	60	20.8	68.6 (34.5)	0	1.3	9.3	0
L. Leg	3	258	568	551	3	42	55	26.4	54.6 (23.2)	0	0.4	12.0	6.6
A794 (3 d.)													
R. Arm	3	416	600	563	6	63	31	14.6	43.3 (35.6)	9.4	23.8	0	8.9
L. Arm	3	534	600	561	7	82	11	23.4	41.2 (18.7)	7.3	20.6	0.2	7.3
C927 (3 d.)													
R. Arm	2	358	532	529	0	67	33	6.7	60.3 (30.7)	4.2	27.7	0.3	0.8
L. Arm	2	305	532	532	0	57	43	1.3	66.3 (37.7)	1.3	30.1	1.0	0
R. Leg	1	228	559	559	0	41	59	0	84.2 (64.0)	0	15.4	0.4	0
L. Leg	1	237	559	559	0	42	58	0	90.7 (64.1)	0	9.3	0	0
C947 (3 d.)													
R. Arm	3	455	503	479	5	86	9	34.1	56.5 (24.2)	2.2	1.5	0.4	5.3
L. Arm	3	364	503	433	14	58	28	43.2	31.6 (16.2)	2.7	1.6	1.6	19.3
R. Leg	1	250	521	521	0	48	52	2.4	87.8 (62.4)	0	0.8	8.0	0
L. Leg	1	306	521	521	0	59	41	8.5	79.8 (48.1)	1.9	1.3	8.5	0
Mean.....		285	543	522	4	48	48	15.1	65.9 (33.3)	1.5	6.7	5.9	4.9

TABLE 6

Late Acute Period (4–6 days)

Analysis of types of neuron damage in samples of motoneuron population supplying arm or leg

ANIMAL AND EXTREMITY	MAXIMAL PARALYSIS	TOTAL CELLS COUNTED	EXPECTED NO. IN SAMPLE	ESTIMATED DESTROYED PLUS ABNORMAL	% OF EXPECTED NO.			% OF REMAINING CELLS					
								Diffuse Chromatolysis (Regressive)		Central Chromatolysis (Recovery)			
					Normal	Abnormal	Estimated Destroyed	Mild	Severe	Mild	Severe	Necrotic	Normal
C944 (4 d.)													
R. Arm	3	440	526	517	2	82	16	14.8	30.0 (9.1)	14.5	38.2	0.5	2.0
L. Arm	2	282	526	525	0	53	47	6.4	25.7 (24.5)	9.2	37.9	0.4	0.4
R. Leg	1	313	565	565	0	55	46	11.2	83.4 (36.1)	0.6	3.8	1.0	0
L. Leg	1	228	565	565	0	40	60	3.9	93.5 (43.9)	0	2.6	0	0
D81 (4 d.)													
R. Arm	2	476	583	578	1	81	18	14.5	72.9 (29.0)	4.2	6.7	0.6	1.1
L. Arm	1	250	583	583	0	43	57	4.8	86.4 (56.0)	0.4	7.6	0.8	0
R. Leg	1	135	511	511	0	26	74	2.2	84.4 (71.8)	0	12.6	0.8	0
L. Leg	0	94	511	511	0	18	82	0	79.8 (70.3)	0	20.2	0	0
C943 (5 d.)													
R. Arm	3	431	522	509	3	80	17	5.1	35.7 (28.8)	13.0	42.7	0.5	3.0
L. Arm	2	357	522	505	3	65	32	6.2	32.1 (23.2)	19.3	37.0	0.6	4.8
R. Leg	1	242	520	520	0	47	53	1.7	60.8 (45.5)	3.3	34.2	0	0
L. Leg	0	210	520	520	0	40	60	0	91.0 (81.0)	0.5	6.6	1.9	0
B332 (6 d.)													
R. Arm	3	318	448	444	1	70	29	17.3	70.1 (23.2)	3.1	7.9	0.3	1.3
L. Arm	2	277	448	448	0	62	38	1.1	80.2 (53.9)	0.1	15.1	3.6	0
R. Leg	4	414	469	437	7	81	12	27.0	41.8 (7.5)	6.8	16.4	0.2	7.8
L. Leg	3	363	469	463	1	76	23	12.9	67.3 (15.7)	4.4	12.9	0.8	1.7
B338 (6 d.)													
R. Arm	3	213	520	503	3	38	59	1.4	17.3 (11.7)	26.8	46.0	0.5	8.0
L. Arm	5	360	520	448	14	55	31	2.8	6.9 (4.4)	35.0	35.0	0.3	20.0
R. Leg	4	316	477	440	8	58	34	5.1	50.1 (39.6)	16.4	16.1	0.6	11.7
L. Leg	5	406	477	365	23	62	15	1.7	25.9 (21.7)	31.3	13.5	0	27.6
Mean.....		306	514	498	3	57	40	7.0	57.7 (34.8)	9.4	20.7	0.7	4.5

TABLE 7

Subacute Period (7–12 days)

Analysis of types of neuron damage in samples of motoneuron population supplying arm or leg

ANIMAL AND EXTREMITY	MAXIMAL PARALYSIS	TOTAL CELLS COUNTED	EXPECTED NO. IN SAMPLE	ESTIMATED DESTROYED PLUS ABNORMAL	% OF EXPECTED NO.			% OF REMAINING CELLS					
								Diffuse Chromatolysis (Regressive)		Central Chromatolysis (Recovery)			
					Normal	Abnormal	Estimated Destroyed	Mild	Severe	Mild	Severe	Necrotic	Normal
B339 (7 d.)													
R. Arm	2	375	568	464	18	48	34	0	3.2 (3.2)	41.1	28.0	0	27.7
L. Arm	1	225	568	519	9	31	60	0	7.6 (7.6)	37.3	32.4	0.9	21.8
R. Leg	1	322	582	575	1	54	45	0	32.3 (32.3)	16.4	48.8	0.3	2.2
L. Leg	2	602	582	535	11	89	0	0	6.2 (6.2)	28.2	54.5	0	11.1
B935 (8 d.)													
R. Arm	3	560	855	684	20	45	35	0	8.0 (8.0)	28.8	30.4	2.3	30.5
L. Arm	2	488	855	671	21	35	43	0	8.4 (8.4)	23.6	22.3	8.0	37.7
R. Leg	0	111	868	842	3	10	87	0	12.6 (12.6)	43.2	11.7	9.1	23.4
L. Leg	1	299	868	800	8	27	65	0	10.0 (10.0)	32.8	32.1	2.3	22.8
B32 (10 d.)													
R. Arm	3	546	775	362	53	17	30	0	1.5 (1.5)	13.4	9.2	0.4	75.5
L. Arm	3	595	775	395	49	28	23	0	0.5 (0.5)	22.2	13.4	0	63.9
R. Leg	2	466	781	471	40	20	40	0	0.9 (0.9)	19.7	12.2	0.6	66.6
L. Leg	2	300	781	644	17	21	62	0	6.7 (6.7)	23.3	22.6	1.7	45.7
B33 (11 d.)													
R. Arm	3	249	589	405	31	11	58	1.6	0.8 (0.8)	12.9	10.9	0	73.8
L. Arm	4	381	589	244	59	6	35	1.1	0.0	6.0	2.3	0	90.6
R. Leg	4	207	408	276	33	18	49	0	0.0	20.4	15.9	0	63.7
L. Leg	5	235	408	258	37	21	42	0	2.1 (2.1)	20.8	12.8	0.4	63.9
B23 (12 d.)													
R. Arm	4	343	419	115	73	9	18	2.0	0.3 (0.3)	7.0	1.8	0.3	88.6
L. Arm	4	295	419	327	22	48	30	12.9	48.9 (17.3)	2.7	4.4	0	31.1
R. Leg	2	181	380	313	18	30	52	11.0	5.6 (3.9)	15.5	30.9	0	37.0
L. Leg	4	290	380	277	43	33	24	10.3	6.9 (1.0)	16.2	10.3	0	56.3
Mean.....		354	623	459	28	30	42	1.9	8.2 (6.2)	21.6	20.3	1.3	46.7

TABLE 8

Subacute Period (14–22 days)

Analysis of types of neuron damage in samples of motoneuron population supplying arm or leg

ANIMAL AND EXTREMITY	MAXIMAL PARALYSIS	PARALYSIS TERMINALLY	TOTAL CELLS COUNTED	EXPECTED NO. IN SAMPLE	ESTIMATED DESTROYED PLUS ABNORMAL	% OF EXPECTED NO.			% OF REMAINING CELLS					
						Normal	Abnormal	Estimated Destroyed	Diffuse Chromatolysis (Regressive)		Central Chromatolysis (Recovery)		Necrotic	Normal
									Mild	Severe	Mild	Severe		
A761 (14 d.)														
R. Arm	3	3	293	478	226	53	8	39	0	2.7	9.2	2.0	0	86.1
L. Arm	4	4	360	478	156	67	8	25	1.6	0.6	7.2	1.1	0	89.5
R. Leg	4	4	334	452	145	68	6	26	0	0	7.5	0.6	0	91.9
L. Leg	4	4	269	452	210	54	6	40	0	0.4	6.3	3.3	0	90.0
B167 (15 d.)														
R. Arm	4	4	367	568	259	54	10	36	0	1.4	13.6	0.8	0	84.2
L. Arm	4	5	444	568	197	65	13	22	0	0.2	14.8	1.4	0	83.6
R. Leg	3	3	293	516	360	30	27	43	0	0.7	30.7	15.0	0.3	53.3
L. Leg	3	3	279	516	389	25	29	46	0	2.9	28.6	22.2	0.7	45.6
B97 (16 d.)														
R. Arm	1		87	467	385	18	1	81	0	1.2	0	2.3	2.3	94.2
L. Arm	1		125	467	349	25	2	73	0	0.0	1.6	0	4.0	94.4
R. Leg	1		245	331	217	34	40	26	0	21.6	14.3	16.3	1.2	46.6
L. Leg	1		89	331	261	21	6	73	0	1.1	7.9	9.0	3.4	78.6
A651 (18 d.)														
R. Arm	1	3	314	477	167	65	1	34	0	0.3	1.0	0	0	98.7
L. Arm	1	2	165	477	315	34	1	65	0	0.6	0	1.2	0	98.2
R. Leg	1	1	360	523	176	66	3	31	0	0	2.2	1.4	0	96.4
L. Leg	1	1	125	523	318	20	4	76	0	0	13.6	2.4	0	84.0
B35 (21 d.)														
R. Arm	1	1	171	732	731	23	0	77	0	0	0.6	0	0	99.4
L. Arm	1	1	140	732	727	18	1	81	0	0	0	3.6	0	96.4
R. Leg	0	0	89	826	822	10	1	89	0	0	2.3	2.3	0	95.4
L. Leg	0	0	125	826	816	14	1	85	0	0	2.4	5.6	0	92.0
C198 (22 d.)														
R. Arm	4	5	375	540	187	65	4	31	0	0	5.3	0.5	0	94.2
L. Arm	4	5	356	540	198	63	3	34	0	0	3.9	0	0	96.1
R. Leg	4	5	384	566	219	61	7	32	0	0	8.9	0.8	0	90.3
L. Leg	4	5	318	566	265	53	3	44	0	0	5.1	0.3	0	94.6
Mean.....			254	540	337	42	8	50	0.1	1.7	7.8	3.8	0.6	86.4

TABLE 9

Early Convalescent Period (35–43 days)

Analysis of types of neuron damage in samples of motoneuron population supplying arm or leg

ANIMAL AND EXTREMITY	MAXIMAL PARALYSIS	PARALYSIS TERMINALLY	TOTAL CELLS COUNTED	EXPECTED NO. IN SAMPLE	ESTIMATED DESTROYED PLUS ABNORMAL	% OF EXPECTED NO.			% OF REMAINING CELLS					
						Normal	Abnormal	Estimated Destroyed	Diffuse Chromatolysis (Regressive)		Central Chromatolysis (Recovery)		Necrotic	Normal
									Mild	Severe	Mild	Severe		
B190 (35 d.)														
R. Arm	5	5	667	811	145	82	0	18	0	0	0.2	0	0	99.8
L. Arm	5	5	591	811	224	72	1	27	0	0	0.7	0	0	99.3
R. Leg	2	5	524	755	243	68	2	30	0	0	2.3	0	0	97.7
L. Leg	1	3	306	755	462	39	2	59	0	0	3.9	0.3	0	95.8
A890 (38 d.)														
R. Arm	2	4	289	514	230	55	1	44	0	0	1.7	0	0	98.3
L. Arm	2	4	248	514	267	48	0	52	0	0	0.4	0	0	99.6
R. Leg	1	3	195	553	360	35	0	65	0	0	1.3	0	0	98.7
L. Leg	1	3	224	553	334	40	1	59	0	0	2.2	0	0	97.8
A690 (38 d.)														
R. Arm	1	2	165	486	325	33	1	66	0	0	1.8	0.6	0	97.6
L. Arm	2	4	228	486	261	46	1	53	0	0	1.3	0	0	98.7
R. Leg	1	3	274	474	211	56	2	42	0	0	2.9	1.1	0	96.0
L. Leg	1	2	82	474	394	17	0	83	0	0	1.2	1.2	0	97.6
A876 (41 d.)														
R. Arm	3	4	331	516	189	63	1	36	0	0	1.2	0	0	98.8
L. Arm	3	4	353	516	165	68	0	32	0	0	0.3	0.3	0	99.6
R. Leg	1	2	186	534	356	33	2	65	0	0	4.3	0	0	95.7
L. Leg	1	2	259	534	283	47	2	51	0	0	2.7	0.4	0	96.9
B188 (43 d.)														
R. Arm	2	4	341	522	192	63	2	35	0	0	2.9	0.3	0	96.8
L. Arm	1	4	375	522	154	71	1	28	0	0	1.6	0.3	0	98.1
R. Leg	2	4	374	529	173	67	4	29	0	0	4.5	0.3	0	95.2
L. Leg	1	2	146	529	396	25	3	72	0	0	6.8	2.1	0	91.1
Mean.....			308	569	268	52	1	47	0	0	2.2	0.3	0	97.5

TABLE 10

Early Convalescent Period (45–49 days)

Analysis of types of neuron damage in samples of motoneuron population supplying arm or leg

ANIMAL AND EXTREMITY	MAXIMAL PARALYSIS	PARALYSIS TERMINALLY	TOTAL CELLS COUNTED	EXPECTED NO. IN SAMPLE	ESTIMATED DESTROYED PLUS ABNORMAL	% OF EXPECTED NO.			% OF REMAINING CELLS					
									Diffuse Chromatolysis (Regressive)		Central Chromatolysis (Recovery)			
						Normal	Abnormal	Estimated Destroyed	Mild	Severe	Mild	Severe	Necrotic	Normal
B186 (45 d.)														
R. Arm	4	4	269	526	274	50	1	49	0	0	1.5	0.4	0	98.1
L. Arm	4	5	334	526	204	62	1	37	0	0	1.5	0.3	0	98.2
R. Leg	1	3	104	520	424	18	2	80	0	0	4.8	2.9	0	92.3
L. Leg	1	3	181	520	340	35	0	65	0	0	0.6	0	0	99.4
B187 (45 d.)														
R. Arm	1	4	318	572	259	55	1	44	0	0	1.6	0	0	98.4
L. Arm	1	1	126	572	450	21	1	78	0	0	2.4	0.8	0	96.8
R. Leg	1	3	207	517	324	37	3	60	0	0	5.8	1.0	0	93.2
L. Leg	0	0	61	517	462	11	1	88	0	0	8.2	1.6	0	90.2
B189 (45 d.)														
R. Arm	2	5	335	493	162	67	1	32	0	0	1.2	0	0	98.8
L. Arm	0	0	92	493	405	18	1	81	0	0	3.3	1.1	0	95.6
R. Leg	1	3	231	530	307	42	2	56	0	0	3.5	0	0	96.5
L. Leg	1	4	286	530	265	50	4	46	0	0	6.3	1.1	0	92.6
B21 (49 d.)														
R. Arm	3	5	294	520	228	56	1	43	0	0	0.7	0	0	99.3
L. Arm	4	5	347	520	177	66	1	33	0	0	1.2	0	0	98.8
R. Leg	2	3	135	529	407	23	3	74	0	0	7.4	2.2	0	90.4
L. Leg	3	5	242	529	290	45	1	54	0	0	1.2	0	0	98.8
B14 (49 d.)														
R. Arm	3	4	250	495	249	50	1	49	0	0	1.2	0.4	0	98.4
L. Arm	4	5	315	495	185	63	1	36	0	0	1.3	0.3	0	98.4
R. Leg	4	5	250	420	176	58	1	41	0	0	2.0	0.4	0	97.6
L. Leg	4	5	326	420	103	76	2	22	0	0	2.5	0.3	0	97.2
Mean.....			235	512	285	45	1	54	0	0	2.9	0.6	0	96.5

second and third days after onset of paralysis, when only about 4% of the total numbers remain normal in appearance, on the average. Similarly low values are maintained through the fifth day, following which a gradual increase in the proportion of normal cells occurs, reaching a plateau in the period between 3 and 7 weeks after onset.

It is interesting that the peak in the proportion of abnormal cells occurs at the time of the lowest values for normal cells, and that a

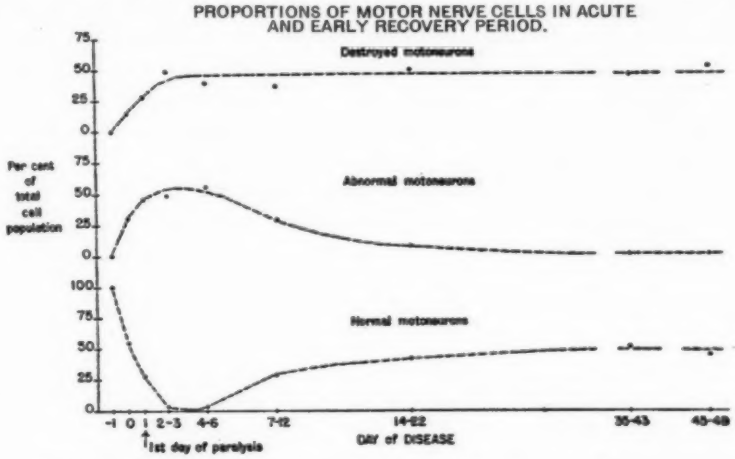

CHART 2. THE CHANGING CHARACTER OF THE MOTONEURON POPULATION AS INDICATED BY THE MEAN CELL COUNTS AT DIFFERENT STAGES.

Each point represents the mean of the motoneuron populations supplying 20 limbs, with an average severity of paralysis of grade 2 (moderate to severe paralysis). Data from Tables 3 to 10. Values for destroyed motoneurons obtained by subtraction of normal and abnormal motoneurons from the total expected number.

steady decline follows which leads to negligible values when the normal plateau occurs. It is important to note that the plateau for destroyed cells is reached rather abruptly, and at a time coincident with peak values for abnormal cells and lowest values for normal cells. This leads to the inescapable conclusion that no appreciable increment of destroyed cells occurs after this time. Moreover, *the increase in normal cells after the second to fifth days must occur by the recovery of abnormal cells.*

Changing proportions of normal cells, and abnormal stages with respect to the total number of remaining cells. In order to obtain more detailed information about the stages of regression and of recovery in infected cells, the changing proportion of normal cells and abnormal stages with respect to the total number of remaining, undestroyed cells was analysed. It was intended that such an analysis should show the progression of cytopathological changes by revealing the time when each stage of cell change reaches its peak in terms of proportion of the remaining cell population. Destroyed neurons were not included, since this portion of the population is essentially negligible after the third day.

In order to classify the pathological stages to be included in the statistical study, a preliminary survey was carried out, based on the following assumptions:

1. The regressive and recovery changes produced by virus action in nerve cells are progressive and follow essentially the same sequence in all cells, although the rates of change may be presumed to be variable.

2. Pathological stages present in large numbers in the acute period of the disease and rare or absent in later periods, represent injury stages.

3. Pathological stages present in large numbers in the subacute and convalescent periods, and rare or absent in the acute stage, represent recovery stages.

The morphological intracellular changes in motoneurons fortunately were sufficiently distinctive in character, as shown in the first section, so that they could be classified in a relatively simple manner. The adequacy of the classification in terms of the above assumptions and of the dynamics of the disease itself appeared to be satisfactorily confirmed by the results to be described. It was found that the predominant cytopathological change in remaining neurons in the earliest preparalytic cases was a diffuse cytoplasmic chromatolysis, largely mild in degree (Table 3, Chart 3, Plate 13, fig. 1). In the early acute paralytic period diffuse chromatolysis of a severe degree increased in prominence, and reached peak proportions between 2 and 5 days. (Severe diffuse chromatolysis, Chart 3, Tables 5 and 6, Plate 13, fig. 2).

Neurons with diffuse cytoplasmic chromatolysis short of complete absence of Nissl bodies were rare after the acute stage, so that progressive diffuse thinning of Nissl bodies may be assumed to identify the regressive

stages due to virus action. After the first 3 days of paralysis, during which diffusely chromatolytic and "necrotic" cells dominate the cytopathological picture, a new morphological appearance of neurons is seen in considerable numbers of cells for the first time. This is the appearance of "central chromatolysis", characterized by an increased density of Nissl bodies in the cell periphery and near the nuclear membrane, but

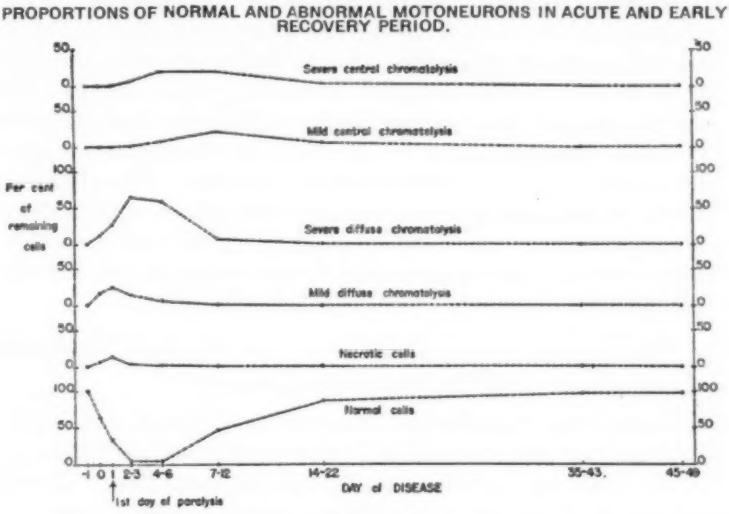
PROPORTIONS OF NORMAL AND ABNORMAL MOTONEURONS IN ACUTE AND EARLY RECOVERY PERIOD.
Severe central chromatolysis
Mild central chromatolysis
Severe diffuse chromatolysis
Mild diffuse chromatolysis
Necrotic cells
Normal cells
Per cent of remaining cells
-1 0 1 2-3 4-6 7-12 14-22 35-43 45-49
1st day of paralysis
DAY of DISEASE

CHART 3

The changing character of the motoneuron population, expressed in proportion of cells in each pathological stage to the total number of remaining cells at each period of the disease. Data in Tables 3 to 10.

with rarefied or absent Nissl substance in the intervening cytoplasm (Plate 13, fig. 3). Whereas the nucleus is generally centrally located in the cell body in cells showing diffuse chromatolysis, it is more apt to be eccentrically displaced in cells showing "central" chromatolysis. The cells showing central chromatolysis are at first relatively few in number and mixed with cells showing diffuse chromatolysis (Tables 5 and 6). By the 7th day after onset of paralysis, however, the latter are rather rare, whereas the former represent the principal pathological cell type except for a few necrotic forms (Chart 3, Plate 13, fig. 4). In

progressively later stages, the Nissl substance is seen in greater amounts in such cells. Thus, the appearance of central chromatolysis with progressively increasing Nissl substance may be assumed to identify the recovery changes in nerve cells injured by virus action. It is interesting that central chromatolysis is first seen when paralysis has ceased to progress and persists in diminishing number of cells, becoming negligible in number after the 3rd or 4th weeks.

Cells which are severely chromatolysed in the acute stage, probably pass through the stage of severe central chromatolysis first, and later that of mild central chromatolysis, before complete recovery of chromophil substance. On the other hand, it is likely that many cells are only lightly chromatolysed at first, and only pass through the stage of mild central chromatolysis before complete recovery. This likelihood seems to be suggested also by the quantitative data showing that the peak proportions of cells showing mild and severe central chromatolysis, respectively, are not sequential but concurrent in time, with the mild stages persisting over a longer period (Chart 3).

III. Damage and Recovery of the Nerve Cell Population as Related to Motor Paralysis and Recovery

The previous sections have illustrated the possibility of studying the injury and recovery changes in the motor nerve cell population in sufficient quantitative detail to make feasible a formulation of several principles which describe rather complex pathological phenomena in a relatively simple manner. The relating of these cellular phenomena to the clinical concepts of paralysis and recovery from paralysis is a matter of much greater difficulty. First of all, the clinical symptom "paralysis" is not easily defined in measurable terms. The normal function of a limb is an exceedingly complex process, in which many parts of the nervous system take part in determining the movement of muscle and bone. The precise timing of a complex sequence of movements and the coordination of action of antagonistic and synergistic muscles are effected by the function of centers in the cerebral cortex, basal ganglia, brainstem, cerebellum, and spinal cord, in response to "voluntary" initiation, and to sensory reflex discharges frequently emanating from the muscles themselves. The central nervous mechanism, however, must exert any influence upon the muscles by acting

through the motoneurons, which constitute the "final common pathway" of CNS motor discharges. Thus, although destruction or damage of motoneurons may be directly productive of loss of muscle function, injury to higher motor centers, which have been shown to be affected in variable degree in poliomyelitis (Bodian, '47), can only be apparent clinically if the "lower" motoneurons are functioning normally. As it happens, the predominant injury in poliomyelitis occurs in the motoneuron population of the spinal cord, so that damage to higher centers either is masked as a rule, or is often too minor in degree to be manifest clinically. Nevertheless, the fact that impaired motor function may result from injury to centers other than lower motor neuron pools makes it difficult at times to assess accurately the degree of weakness or "paralysis" in poliomyelitis. For example, the spasticity of early poliomyelitis in monkeys may be so extreme as to make it difficult for animals to climb, although motoneurons are quite intact (Bodian, '47). Manual examination of extremities in more detail, however, reveals no lack of power, but only muscular rigidity as the limiting factor in movement. The rigidity, of course, disappears in those muscles where paralysis due to lower motor neuron damage becomes severe. It is thus evident that loss of muscular power rather than inadequacy of motor performance is the best index of motoneuron damage in the acute stage, and in later stages atrophy of muscles can be used as a confirmatory sign of motoneuron destruction.

The total potential power of contraction is normally rarely called into play, and in fact it is obvious that only a small fraction of the potential muscle power is necessary for ordinary motor activities. When a measure of motor function is desired, this is usually obtained by assessing the potential power of muscles or muscle groups by means of ergographs or by means of less accurate arbitrary standards which involve the degree of performance against the resistance of gravity. Since the available power of normal muscles is determined by the general physical condition of the individual, and varies with fatigue, exercise, nutritional state, etc., it is obvious that the motor function of the CNS, or even of the muscle itself cannot be accurately quantitated over a period of time without control of these factors When all these factors are considered, the potential power of some single muscles or of single synergistic muscle groups can be measured with a fair degree of

accuracy. Unfortunately, however, the motoneurons supplying individual muscles or small muscle groups cannot be accurately localized in the spinal cord except by experimental means, and their localization in an infected spinal cord is even more difficult. As we have earlier indicated, the motoneuron population supplying an entire limb can be more readily identified, and a reasonably accurate sample of the population can be counted and classified into pathological stages. We have therefore accepted the difficulty of accurately assessing the motor function of an entire limb and have used a system of grading which is not over-refined but which appears to be adequate for our purposes. The method has been described in one of the introductory paragraphs of this report. It must be emphasized here, however, that the grades of function are arbitrary and represent quantities of increasing magnitude but of unknown value.

The Role of Motoneuron Destruction

In view of the relative crudeness of muscle testing, the first question which must be asked is how closely can motor function be correlated with motoneuron destruction in the early convalescent period. At this time, between about one and three months after onset, the simplest possible relation between the size of the remaining motoneuron population and the degree of residual weakness may be obtained, since most of the remaining neurons are normal in appearance, and probably in function. Moreover, hypertrophy of muscles, which may reach considerable proportions in partly paralysed limbs after the third month, has not as yet had a chance to affect the relation between residual motoneurons and residual muscle function to too great a degree. It is not uncommon, for example, to find an increase in mass of muscle of a partly paralysed limb from about one-half of that of its normal mate to almost normal dimensions between the third and sixth month.

Table 12 shows the relation between degree of paralysis and the percentage of surviving motoneurons of 78 paralysed limbs from 20 animals (Tables 8 to 11) killed between three weeks and three months after onset of paralysis (see also Table 15, D; and Chart 4, D.) The average grade of severity of paralysis of all limbs was roughly 2 at the height of paralysis, and 3 at the time the animals were killed. In all of these animals the surviving motoneurons were all or almost all normal

TABLE 11

Relation of Paralysis to Destroyed Motoneurons in Convalescent Period

ANIMAL AND EXTREMITY	DURATION OF PARALYSIS IN DAYS	MAXIMAL PARALYSIS IN ACUTE STAGE	PARALYSIS TERMINALLY	TOTAL CELLS COUNTED	EXPECTED NO.	% ESTIMATED DESTROYED
C903	31					
R. Arm		1	2	174	500	65
L. Arm		1	2	229	500	54
R. Leg		0	0	0	600	100
L. Leg		0	0	0	600	100
C437	57					
R. Arm		1	3	173	442	61
L. Arm		1	2	89	442	80
R. Leg		0	0	53	437	88
L. Leg		0	0	19	437	96
A505	75					
R. Arm		2	4	244	461	47
L. Arm		2	4	242	461	48
R. Leg		1	1	42	317	87
L. Leg		1	1	116	317	63
A506	72					
R. Arm		1	2	126	440	71
L. Arm		1	2	133	440	70
R. Leg		1	1	51	258	80
L. Leg		1	2	57	258	78
A713	84					
R. Leg		1	2	79	513	85
L. Leg		1	5	326	513	37
A714	84					
R. Arm		2	5	293	549	47
L. Arm		2	5	381	549	31
R. Leg		1	5	302	505	40
L. Leg		1	5	343	505	32
A956	93					
R. Arm		4	4	247	575	57
L. Arm		4	5	379	575	34
R. Leg		0	1	113	745	85
L. Leg		0	1	60	745	92
A394	101					
R. Arm		0	0	64	565	89
L. Arm		4	4	319	565	44
R. Leg		5	5	392	516	24
L. Leg		4	4	239	516	54

in appearance. The mean and the range of the percentages of surviving cells are shown in the motoneuron population of limbs of specified grade at the time of death. Of special interest are the values for

TABLE 12

Relation between Severity of Paralysis and Destroyed Motoneurons in Early Convalescent Period†

CLINICAL GRADE WHEN KILLED‡	PER CENT OF MOTONEURONS DESTROYED		NO. LIMBS
	Mean	Range	
5	34	18–54	22
4	44	28–57	17
3	62	42–80	10
2	70	52–85	12
1	80	63–92	8
0	91	81–100	9
Total			78

† Animals killed 3 weeks to 3 months after onset.
‡ Average clinical grade at height of paralysis—about 2.
Average clinical grade when killed—about 3.

TABLE 13

Relation between Severity of Paralysis at its Height and Destroyed Motoneurons†

CLINICAL GRADE AT HEIGHT OF PARALYSIS‡	PER CENT OF MOTONEURONS DESTROYED		NO. LIMBS
	Mean	Range	
5	28	18–43	4
4	35	18–57	24
3	41	23–58	12
2	42	0–74	18
1	62	26–87	44
0	90	81–100	12
Total			114

† Animals killed 7 days to 3 months after onset.
‡ Average clinical grade at height of paralysis—about 2.

limbs graded as normal, and as completely paralysed (5 and 0), since these clinical grades, and especially the latter, are the most accurately determined. It will be noted that of 22 limbs graded as "normal" in

function at the time of death, none showed less than 18% destruction of motoneurons, and the average value was 34%. It, therefore, appears that as much as a third of the motoneuron population may be destroyed without detection of weakness of the limb by our method of examination, which includes observation of climbing, running, or jumping, careful manual examination, and inspection for evidence of atrophy (see also Pette, Demme, and Környey, '32; page 245). Our earlier observations suggest that this figure for "normal" limbs may be high for many non-paralytic cases, but it is not high for paralytic cases regardless of the "mildness" of the paralysis. Of the 22 limbs graded as "normal" between the first and third months, 3 were never noted to be paralysed, 10 were paralysed to the degree 4 at the height of paralysis, and the remaining were distributed among grades 3, 2 and 1 at the height of paralysis.

At the other extreme it was found that 9 of 11 limbs completely paralysed in the acute stage, or showing only traces of muscular activity, failed to recover any function whatever. This agrees with general experience in human cases, in which it is found that the possibility of any recovery whatever is very low in extremities completely paralysed in the acute stage. In only 2 of our 9 cases of total limb paralysis, however, was the destruction of motoneurons complete in the sections examined. The average loss was 91%, which suggests that a residual population of about 10% of motoneurons is incapable of producing clinically observable movement in some cases. In other cases, graded as 1 when killed, a residual population no larger or very little larger was capable of producing movement because it was concentrated largely in an area supplying a small muscle group, usually the toe or finger flexors, which often was the last group to be involved.

The proportion of destroyed motoneurons in the intervening grades (4 to 1), at the time the animals were killed, shows a good correspondence with the degree of paralysis. Although there is considerable overlap in the ranges of adjacent groups, this is not surprising in view of the difficulties of clinical grading and the role of variation in distribution of destruction, to be discussed. The demonstration of a general proportionality between motoneuron destruction and degree of paralysis in the early convalescent period is, of course, confirmatory of an established fact which can be verified by a much easier survey of spinal cord

sections from selected cases. The larger quantitative experience, however, makes possible more accurate analysis of details with an assurance that cannot be obtained by an unsystematic survey of spinal cord sections.

With a degree of confidence in the validity of the method of relating motor function to the status of the motoneuron population, engendered by the above described results, we can now proceed to examine in the same animals the relationship between motoneuron destruction and clinical grade at the height of paralysis in the acute period. The values given in Table 13 show the percentages of surviving motoneurons in animals killed between 3 weeks and 3 months after onset, as related to limbs of specified clinical grade at the time of the height of paralysis in the acute stage. An additional 36 limbs (Tables 7 and 8) from animals killed after the seventh day are added to those of Table 12. Since it was shown in the previous section that negligible death of motoneurons occurs after the height of paralysis is reached, it may be assumed that the number of motoneurons found destroyed at any time after 7 days is the same as that which would have been observed if the animal had been killed at the height of paralysis. It will be noted at once that in animals with the severest grade of paralysis (0) at the height of paralysis, the proportion of destroyed motoneurons is the same as it is in animals with the same clinical grade when killed in the early convalescent period (Table 12), as is to be expected. The limbs in other grades at the height of paralysis, however, show a consistently lower mean value of destroyed motoneurons than limbs of comparable grade in the early convalescent period. In grades 1, 2 and 3 the difference is about 20% in each. This indicates that a sizable component of the paralysis of the acute period cannot be accounted for by motoneuron destruction. That this component may be very large in some cases is strikingly shown by the lowest values in grades from 1 to 5, all of which fall below 26, whereas the lowest values in Table 12 follow a stepwise order from 18 to 63 per cent, consistent with the mean values

It is plain, therefore, that most of the remaining weakness observed in the early convalescent period can be accounted for on the basis of motoneuron destruction alone, whereas the paralysis observed in the acute stage is also in part the result of other factors. One of these factors is probably the reversible injury of motor nerve cells, since it

was shown in the preceding section that many motoneurons may be severely injured and yet recover, as far as microscopic structure is concerned, before the early convalescent period

The Role of Reversible Motoneuron Injury

With the knowledge that extensive reversible changes occur in motoneurons in the acute period, we have attempted an analysis of their role in the production of the recoverable component of paralysis.

TABLE 14

Relation between Severity of Paralysis and Pathological Status of Motor Nerve Cells in Acute Period (0 to 12 days)

CLINICAL GRADE WHEN KILLED	1		2		3		NO. LIMBS
	% of Cells Destroyed		% of Cells Destroyed, Necrotic or Completely Chromatolysed		Column 2. Corrected to Incude ½ of Cells Showing Severe Chromatolysis†		
	Mean	Range	Mean	Range	Mean	Range	
5	18	0–43	28	5–68	32	12–72	29
4	30	12–49	39	18–60	46	19–69	9
3	31	10–59	44	23–77	56	28–82	18
2	38	0–60	59	36–90	70	45–91	15
1	60	40–89	79	63–95	87	70–97	16
0	82	60–91	94	90–97	96	91–98	5
Total							92

† This gives severely chromatolysed cells the functional value of half as many normal cells.

The motoneuron populations of 92 limbs were studied in animals killed between the preparalytic period and 12 days after onset of paralysis. As can be seen in Table 14, column 1, and in Chart 4, A, the average proportion of destroyed cells alone is much less for each grade of clinical function than is the case in the early convalescent period when the morphological status of the motoneuron population is stabilized. (Table 12; Chart 4, D). In Table 14, column 2, the proportion of all cells which were considered as very likely to be non-functional is given for limbs of each clinical grade. These cells include estimated destroyed cells, cells which we have previously defined as "necrotic", but which still have recognizable nuclei, and cells which are completely

chromatolysed, that is, have no remaining Nissl substance in the cytoplasm. These proportions, also shown in Chart 4, B, compare well with those found in the "stable" motoneuron population of the early convalescent period (Table 12; Chart 4, D), with the greatest discrepancy seen in limbs of grades 2 and 3. Since it was thought that some cells graded as "severely chromatolytic" could be functionless

RELATION BETWEEN SEVERITY OF PARALYSIS IN ACUTE STAGE (0 TO 12 DAYS) AND PATHOLOGICAL STATUS OF MOTONEURONS.

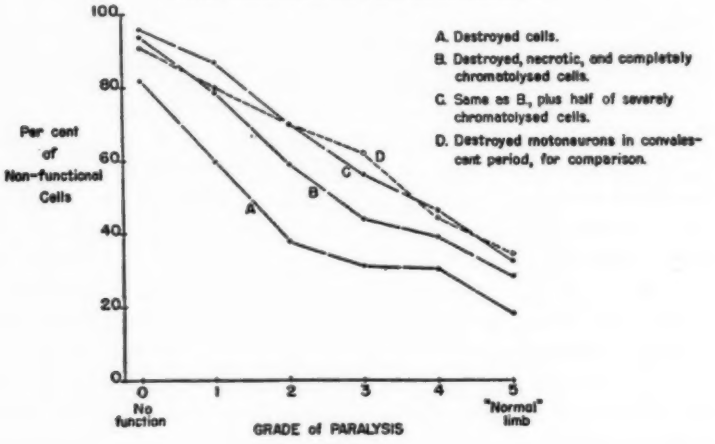

CHART 4

See page 51 for detailed explanation. The lines A, C, and D correspond to the correlation tables in Table 15, and show the trends of mean proportions of "non-functional" motoneurons in relation to functional grades. Since the quantitative value of each clinical grade is unknown, as explained on page 44, the lines are not to be considered as accurate curves, since the slopes are unknown.

The grades of paralysis shown below are explained on page 8. Grade 5 represents normal or almost normal limbs, and grade 0 completely paralysed limbs.

also, this assumption was then tested as well. It was found that when half of such cells were counted as non-functional, and added to those included in Table 14, column 2, a result was obtained which fitted well with the comparison of motoneuron populations and clinical grade of the early convalescent period. (Compare Table 12 and Table 14, column 3; and Chart 4, C and D). This result brought out a fact previously unrecognized by us, namely that severely chromatolysed cells were relatively more numerous in the "middle" grades of paralysis (2 and 3).

The correspondence between lines C and D in Chart 4 is of considerable interest in suggesting that the function of an infected neuron is lost in the stage of injury change which we have described as "severe

TABLE 15

Grade of Limbs in Poliomyelitis Cases in Relation to "Non-Functional" Motoneurons†

GRADE OF LIMBS IN ACUTE STAGE (0–12 D.)	0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–100	
A. % of Cells Destroyed											
5	7	11	8	1	2						
4		2	2	4	1						
3	1	5	5	3		4					
2	1	2		5	5	2	1				r = 0.76
1					3	7	1	3	1		
0						1	1		2	2	
C. % of Cells Destroyed, plus Necrotic Cells, Completely Chromatolysed Cells, and ½ of Severely Chromatolytic Cells											
5	3	4	9	6	3	2	1	1			
4			2	3		3	1				
3			1	4	4	3	4	1	1		
2				1	2	3	4	3	2	1	r = 0.82
1							1	5	6	4	
0										5	
D. % of Cells Destroyed											
GRADE OF LIMBS IN SUB-ACUTE STAGE (21 D.–3 MO.											
5		1	4	12	4	1					
4			2	3	8	4					
3					1	4	3	2			r = 0.86
2						2	4	4	2		
1							1	3	3	1	
0									6	3	

† The frequency numerals in the tables refer to the number of limbs in each category. Grade 5 = normal; grade 0 = complete paralysis.

chromatolysis", and that cells in stages of more severe change are functionless. This supposition is in agreement with findings from individual cases described in Section II. Moreover, the great disparity

between values of C and those of A, especially in the "middle" clinical grades, suggests that a large component of the lost function in the acute stage may be due to the reversible injury represented by the stages of severe chromatolysis. Cells in these stages are very numerous between the fifth and twelfth days, when significant numbers of cells are no longer dying off.

Table 15 shows these relationships in the form of a correlation table, with A, C, and D corresponding to the trends in mean values shown in lines A, C, and D of Chart 4. An obviously high degree of correlation between grade of limb function and destroyed motoneurons is shown in the early convalescent period (D), with a correlation coefficient, r, of 0.86.[2] In the acute period the correlation, although high also, is somewhat less (A), with a shift in the direction of less motoneuron destruction in each grade than shown in (D). This means that cell destruction alone cannot completely account for the degree of paralysis observed, although it is an important factor. In (C) the effect of including necrotic cells, completely chromatolysed cells, and half of the severely chromatolysed cells in the category of "non-functional" is shown. The relation between grade of function and proportion of non-functional cells is much closer to that found in the early convalescent period (D). The discrepancy is very likely due in large part to the greater difficulty of accurately assessing grades of function in the acute period. In the latter stages, for example, the presence of asymmetrical atrophy of limbs is a helpful adjunct in the assessing of function and probably prevents certain errors of grading to some extent.

The Role of Distribution of Cell Injury and Destruction

It was expected at the beginning of this study that great difficulty would be encountered in comparing the pathological status of the motoneuron population and the function of the corresponding limb as a whole. Spotty paralysis in a limb is often observed, with obvious paralysis of one muscle group and very little if any, in another group in the same limb. Similarly, one frequently observes severe destruction of motoneurons on one side of a spinal segment, or part of one, and little if any destruction of adjacent segments. Although these occur-

[2] A correlation coefficient of 1 represents complete association; one of 0 represents no association.

rences are striking, they are by no means the rule, since a relatively homogeneous distribution of pathological effect is also commonly seen. Moreover, in our experience, the spotty distribution of pathological change rarely results in the selection of a single functional muscle group for destruction, but usually has a segmental character, so that alternating regions along the cephalocaudal axis are affected severely or moderately. As is well known, segmental lesions, such as follow spinal root section, produce diffuse and unspectacular weakness because spinal roots of the limb areas supply antagonistic groups, and because no important limb muscle groups are supplied by only one spinal root. Consequently, the function of the limb as a whole has been a surprisingly good average indicator of the state of most of its constituent muscle groups. Moreover, as was shown in Section 2, paralysed animals have such a widespread distribution of motoneuron damage in the entire motoneuron population, that it is hard to believe that any limb muscle group can escape some degree of injury, although some loss of function may be below the clinical threshold.

Nevertheless, of course, single muscles or muscle groups occasionally are hard hit by an unusual localization of neuron damage and destruction, or conversely a muscle or muscle group may be spared to an unusual extent. In the monkey the muscles most commonly spared in otherwise completely paralysed limbs are the flexors of fingers or toes, and a correspondingly intact group of motoneurons can be found in such cases in the posterolateral cell column in the two lowest segments of the spinal cord enlargements. In several convalescent cases we have found such isolated sparing of motoneurons of this group in the brachial region to be associated with severe flexion contracture of the fingers, due to unopposed action of the flexor muscles. Such contractures did not occur in arms corresponding to spinal cord enlargements in which this motor cell group was completely or almost completely destroyed.

It is interesting that Elliott ('45) has confirmed earlier workers in describing a pattern of neuron destruction in convalescent and chronic human cases in which the ventrolateral cell groups of the anterior horn are most commonly spared, and most constant injury occurs in the region of the commissure and in the center of the horn (Blanton, '17). Elliott, however, interprets this as indicating a concentric spread of infection from a dorsomedial point in the cross-section of the spinal

cord. From a study of acute cases as early as the preparalytic period one arrives at a rather different interpretation of his findings. Although we have observed a tendency for early inflammatory lesions to be located dorsomedially, especially in the vicinity of branches of the anterior spinal vessels, we have seen no suggestion in earliest preparalytic cases that the first neuron lesions are located dorsomedially or spread concentrically. As was mentioned in Section 1, the earliest changes in motoneurons, without surrounding inflammatory changes, may be seen at the lateral periphery of the anterior horn or in any other position. Spread of virus seems to be so rapid and general that in the early paralytic period most anterior horn cells in the enlargements show evidence of infection. Observations made in the chronic stage cannot reveal the generalized character of the infection in the motoneuron population, since remaining neurons are completely recovered as a rule and may seem not to have been involved.

In our convalescent and chronic cases the pattern of neuron destruction is quite variable, but there is a tendency for motoneurons in the periphery of the anterior gray columns to have a greater chance of survival. Not only the ventrolateral group, emphasized by Elliott as preferentially spared, but also the most medial cells are more often persistent than centrally located cells in the anterior columns of the enlargements. This is also Horanyi-Hechst's finding in an extensive study of human cases ('35). Moreover, the neurons bounding the enlargements in the longitudinal plane are also more resistant to destruction than those in the limb areas, as is well known. In the monkey, sacral, thoracic and upper cervical motoneurons are in this category (Pette, Demme, and Környey, '32). Elliott's concept of concentric spread of lesions from the commissural region must therefore be questioned, since his findings indicate only a tendency of preferential sparing of peripheral neurons rather than the direction of spread of the pathological process.

In many cases, not even the above-mentioned pattern of sparing of motoneurons is seen. Frequently one observes severe destruction of lateral groups with complete sparing of medial groups, and vice versa. Moreover, in some cases foci of extensive cell destruction alternate irregularly with areas of sparing, whereas in others a rather homogeneous destruction of nerve cells is seen with not a single large area of

motoneuron disappearance. In such cases, there is no suggestion of a "pattern" of preferential cell destruction.

Finally, it is problematical whether the tendency for greater paralysis of some muscle groups and more common sparing of others can be correlated precisely with corresponding tendencies for greater or less "susceptibility" in their motor nerve cell "groups". For example, a given proportion of motoneuron destruction in a functional group with high innervation ratio may produce a quite different result than the same proportion in a group with a low innervation ratio. When relatively few motoneurons supply a large muscle, destruction of a given proportion may denervate a relatively large mass of muscle, whereas equivalent destruction in a functional center supplying a small muscle with a high innervation ratio would denervate a relatively small mass. Moreover, the greater compactness of a functional motoneuron group may prejudice the chances for survival of a sufficiently large number of cells of a functional pool to produce adequate function. Unfortunately our knowledge of nerve-muscle relationships is not sufficient to give all of these factors their proper weight in the determination of the pattern of paralysis.

Distribution of Lesions in Non-Paralytic Cases and in Monkeys Infected with "Mild" Strains

In previous reports (Sabin and Ward, '41; Bodian and Howe, '41a) the wide variation in the extent of lesion formation in non-paralytic attacks of poliomyelitis has been described. In the mildest of such cases in the rhesus monkey not only may the cerebral lesions be more limited than they are in paralytic cases but also lesions may be found in only a few segments of the spinal cord or may be quite absent in the spinal cord enlargements although present in the hindbrain (Bodian and Howe, '41a, case A922; no lesions in cord segments C5 to T1, and L4 to S1). This is in striking contrast with the paralytic cases described in this report, in which hardly a section in the cord enlargements was free of lesions. As a matter of fact, most non-paralytic cases show as extensive distribution of lesions in the spinal cord as do paralytic cases, and differ from the latter not in the number of motor nerve cells invaded but rather in the severity of the injury to invaded nerve cells and in the number destroyed. Most non-paralytic cases and cases

with very slight weakness are indistinguishable, as far as cord pathology is concerned, from the part of the cord of "severe" cases which supplies a spared extremity.

It is interesting, moreover, that animals infected with "mild" poliomyelitis strains show a similar extensive distribution of lesions and a high proportion of invaded motoneurons. For example, the Frederick strain, isolated from a human throat swab in 1944 in Baltimore (Howe and Bodian, '47), exhibited the mildest paralytic cases in our experience in animals inoculated with virus from the first monkey passage. These animals nevertheless showed lesions differing in no way from those of "severe" strains in extent of distribution or in character, except that invaded cells usually showed less severe cytopathological changes and fewer were destroyed. Inflammatory changes were also much less severe as a rule, as might be expected with less cell destruction. Yet in some sections all motoneurons on one side were destroyed, so that the local picture resembled that seen in animals with prostrating paralysis. In one case (C59) killed 4 days after slight paralysis was suspected in one arm and one leg, and showing no progression of paralysis, only 4% of the expected number of normal cells was found in a sample of 79 sections through all segments of brachial and lumbar cord enlargements. Most of the motoneurons were in the stage of mild chromatolysis, (59% of remaining cells), and 21% of the expected number were destroyed (Plate 14).

The extensive motoneuron invasion with "mild" strains like the Frederick agrees with the finding that a pool of spinal cords from animals inoculated with virus from the first monkey passage gave a titer of about 1×10^{-6} in monkeys inoculated intracerebrally. This compares well with the titers obtained with pools of "severe" strains under the same conditions, and suggests that optimum virus titers, as well perhaps as virus dissemination, in the nervous system is not dependent on extensive destruction of host cells.

DISCUSSION

Cytopathological Changes in Motoneurons. The finding of progressive chromatolytic changes in motoneurons in the acute stage agrees with earlier observations made by Hurst ('29) and others, in both monkeys and man. Sabin and Ward ('41) have illustrated the probable se-

quence of changes in a manner similar to that shown in Plate 1 of this report, and from examination of non-paralytic cases have suggested "that a cell attacked by poliomyelitis virus perhaps need not invariably be irreversibly damaged". The quantitative survey of accurately timed stages in the present study leaves no doubt that this occurs, and shows in fact that in some cases a high proportion of cells are chromatolysed and subsequently recover. Of special interest is the fact that cells which are chromatolysed by virus activity are either quickly destroyed during the first few days of the disease or undergo slower recuperative changes leading to complete morphological recovery in a period of about 1 month. Both Hurst ('29) and Sabin and Ward ('41) have commented upon the normal appearance of most nerve cells after this time, but our larger series of cases shows that this essentially complete recovery of motoneurons is the rule. Moreover, the stages of morphological recovery can be identified, using the Nissl substance as an indicator, and studying cell populations quantitatively in a sequence of clinical stages.

Comparison of Humans and Chimpanzees with Rhesus Monkeys. It is evident that numerous difficulties prevent an analysis of human material in as adequate detail as is permitted in experimental animals. Among these, first of all, is the problem of determining the time of onset of pathological changes. In monkeys the use of "severe" passage strains produces a fairly abrupt onset, so that it is possible to time the early stages of the disease accurately (Bodian and Cumberland, '47). The onset of the disease in humans is more often insidious, or of the "dromedary" type, and at times no clinical symptom clearly marks a definite stage of the histopathological process. Moreover, the important preparalytic stage, and the earliest paralytic stage, is not available in autopsy material. Finally, autopsy material is often poorly preserved, so that postmortem changes mask important cytopathological changes.

In spite of the afore-mentioned difficulties, a study of the changes in human spinal cords as compared with those worked out in detail in the monkey has proved to be interesting and instructive. Our own available material includes the spinal cords of 16 fatal human cases, prepared as much like our experimental material as possible. Six of these cases showed such overwhelming destruction of anterior horn cells at

all levels that they were of no value in studying sequential changes in these cells. In the remaining ten cases the disease had had a duration of from 1 to 2 days in the earliest to about 8 to 12 days in the latest, and of these cases only about half showed relatively good postmortem tissue preservation.

Only outstanding features of the histopathological process could be used to compare the disease with that in the monkey. In the earliest human case available (H35) the disease had had a duration of only 1 to 2 days before death ensued. This case was indistinguishable in essential particulars from monkey cases of similar duration. The predominant pathological stage in motoneurons consisted of diffuse chromatolysis, with many cells completely chromatolysed, necrotic, or undergoing early neuronophagia. A similar sequence of changes in motoneurons in a child of 4 years who died 30 hours after onset of the disease was described and well illustrated by Craw ('31). Three cases were available with a disease duration of about three days (H33, H36, H37). In all three cases there was definite recession of the destructive process, as far as the spinal motoneuron population was concerned. Active neuronophagia was less conspicuous, and chromatolytic cells less numerous than in the earlier case. In one of these cases, central chromatolysis, as well as diffuse chromatolysis, was seen in some of the lumbar segments.

It is interesting that active neuronophagia was uncommon in all five of the cases with a duration of disease longer than 3 days. The predominant pathological form seen among motoneurons in these cases was that of central chromatolysis, although numerous necrotic cells were also in evidence. In the case with longest duration (H34), with disease onset about 8 to 12 days before death, the first paralysis was noted 5 days before death. In this case, cells with typical central chromatolysis were abundantly seen, greatly exceeding in number the pathological stage with next greatest frequency of occurrence, namely persistent necrotic forms. Diffusely chromatolytic cells were infrequent in occurrence, and not only neuronophagia but also the cell clusters which persist for a period after neuronophagocytosis has been completed were conspicuously absent. The motoneuron illustrated by Goodpasture ('41) well illustrates this stage of the disease (central chromatolysis—fig. 7, page 121).

Although the pathological process in man, including the time course, is remarkably similar to that in the monkey, certain important differences were observed. Except for the fulminating case (H35), the other human cases appeared to be older by one or several days than suggested by the time of recorded onset of paralysis. This suggests that the pathological process in man as a rule does not start as abruptly as in severe monkey cases inoculated with large doses of virus, but perhaps starts earlier than indicated by the clinical history. It is quite possible, for example, that the pathological process in the nervous system in man may begin at the time of the first hump in "dromedary" cases. In such cases the delay in onset of paralysis would perhaps mean a more gradual spread of the destructive process in the cord as compared with the more commonly fulminating disease in artificially inoculated experimental animals. Moreover, the virus strain may play a determining role, since even in rhesus monkeys the disease is more insidious in onset when "mild" virus strains are used than when "severe" strains are involved.

The impression was obtained that in the human cases, as compared with monkeys, the motoneurons were more likely to be completely spared if they were not destroyed. This was suggested by an apparently greater proportion of normal cells in the early recovery period, but the human cases were too few in number to permit safe generalization.

Our chimpanzee material examined for comparison with the rhesus monkey was not extensive, but showed close similarity to analogous cases of rhesus poliomyelitis (compare Plate 15, figs. 1 to 6, and Plate 8, figs. 1 to 6).

Comparison of Poliomyelitis with Other Neurotropic Virus Diseases. Although extensive material was not available for comparing the sequence of cytopathological changes in motoneurons in various neurotropic virus diseases, several interesting similarities were found. For example, the rapidly appearing and progressing diffuse chromatolysis characteristic of early poliomyelitis is also found in rhesus monkeys infected with equine encephalomyelitis virus, and with neurotropic yellow fever virus. In all three diseases the diffuse chromatolysis of Nissl substance is the first occurrence in spinal motoneurons, and may often be unaccompanied by any inflammatory

changes in the vicinity. The time course of diffuse and central chromatolytic changes observed in poliomyelitis was also found to occur in essentially similar fashion in equine encephalomyelitis and in neurotropic yellow fever, as is illustrated by the representative figures in Plate 16, figs. 1 and 2, and Plate 17, figs. 1 to 3.

Statistical Findings. A surprising finding in this study was the very high incidence of pathological change in the motor nerve cell population, indicating an almost universal dissemination of virus in the spinal motor cell population in paralysed individuals. In 40 separate limb regions of 11 animals killed 2 to 5 days after onset of paralysis, the percentage of normal cells in motoneuron populations averaged between 3 and 4%. Of these limb regions, 7 supplied extremities either clinically normal or showing only mild paralysis, and these showed an average of about 10% of normal motor cells in the total cell populations. Five of these extremities were in animals killed on the 5th day and in which there was no possibility of progression of paralysis. These findings indicate that in some cases reversible changes may affect the majority of the motoneuron population. Additional strong evidence in this regard comes from the finding that whereas normal cells increase from an average proportion of 4% to 50% during the first month after the height of paralysis, destroyed cells do not increase noticeably during this period. In confirmation of the latter point, moreover, necrotic cells not yet absorbed are negligible in numbers after the third day.

Another important finding concerns the overall death rate of motor nerve cells in relation to the maximal degree of paralysis in the disease. The average proportion of destroyed cells, we have shown, does not vary appreciably after the height of paralysis is reached (2 to 3 days after onset of paralysis). This proportion of the total motoneuron population is about 47%, and represents also the grand average of the 120 limb populations examined in this period. Since, on the average, all but 4% of motoneurons are involved in the acute stage, the average proportion of injured nerve cells which are destroyed, or the average "case fatality rate", is almost 50%. The range in our series of cases from the second to the fifth day varies from 12% to 91%. One can therefore state that in this series as a whole there was a 50% probability that an invaded motor nerve cell would be destroyed by virus activity.

The finding of a generally high incidence of infection of motoneurons obviously indicates that the disease is not necessarily arrested by failure of dissemination of virus to the entire motoneuron population, since the "exposure" of cells as shown by pathological changes is almost universal in some cases. This finding also brings up the important but difficult question of what factors produce the arrest of the disease in some motoneurons and not in others. In the present state of our knowledge of the virus-neuron reaction, an adequate consideration of the factors which may determine cell death or cell recovery is impossible, but two lines of speculation suggest themselves. First of all, it has been shown that experimentally induced changes in the metabolism of nerve cells can greatly increase their resistance to destruction by poliomyelitis virus (Howe and Bodian, '41). It is possible that variation in the normal state of the susceptible nerve cell at the time of infection may be sufficient to account for variation in the outcome of infection. On the other hand, the rapid decline of virus concentration in infected spinal cord in the acute stage (Bodian and Cumberland, '47) suggests the possibility that immune factors begin to operate early in the disease. Since the monkey motoneuron population initially consists entirely of non-immune cell individuals, arrest of the disease by antibody factors would require a very rapid mobilization of such factors locally, perhaps in the nerve cell itself. It is conceivable that variations in the rate of virus multiplication or the speed of mobilization of such immune factors might determine the outcome of cell infection. Finally, as was mentioned before, it is clear that variations in the properties of different virus strains, as well as host factors, play a role in this problem.

The high incidence of infection of motor nerve cells in paralytic cases and the fact that second paralytic attacks may occur in individuals exposed to heterologous virus strains suggest too that it is possible for a recovered nerve cell to be re-infected by heterologous virus. In several rhesus monkeys in which second paralytic attacks had occurred after an initial attack with heterologous virus several months previously, and involving severe paralysis with atrophy of at least one extremity, we found extensive acute changes in motoneurons. We have also observed two instances in which the second attack was non-paralytic, and in which widespread chromatolysis in motoneurons

gave conclusive evidence of a fresh infection due to the second inoculation. In such cases, the conclusion is inescapable that nerve cells previously infected and recovered were re-infected by heterologous virus. Presumably reinfection of cells with homologous virus is prevented by the presence of a corresponding antibody "barrier" which precludes access of virus to the cell.

Relation of Changes in Motoneurons to Paralysis and Recovery. In an earlier report we have listed several possible pathological factors which may be concerned with the interference with motor function in poliomyelitis and with recovery (Bodian, '46). It was shown that interference with motor performance, especially preceding the onset of lower motor neuron weakness, could be the result of severe lesions which frequently occur in brainstem centers. Of these, the reticular formation damage is the most likely source of the spasticity which occurs in some cases, and the damage to the fastigial nuclei of the cerebellum, and to the associated vestibular nuclei, the probable source of the tremor and ataxia which are sometimes manifest. In this report, we have dealt only with the two symptoms due to lower motor neuron injury, namely decrease of muscle power, which we have referred to as paralysis, and muscle atrophy. It seems reasonably certain from the evidence now presented that the pathological and recovery changes in motoneurons alone can account for most of the paralysis and its early recovery, respectively. Other factors probably play a secondary or even negligible role, although the possibility that they play a greater role in special cases cannot be excluded from the evidence at hand. The slow, but sometimes significant, increase of power in paralysed limbs after the third month has not been studied in detail by us because of the small numbers of animals available. Since the morphological and probably functional status of the motoneuron population does not seem to change significantly after the first and second months, such recovery as occurs after this time probably is the result of slower compensatory changes in the entire neuromusuclar mechanism. In a small number of monkeys studied for as long as a year after onset of paralysis, the late recovery was regularly asociated with increase in size of previously atrophied muscles. Since this process is continuous and apparent for as late as 1 year, it most likely represents a gradual compensatory hypertrophy of muscle fibers with intact innervation rather than the

result of reinnervation of denervated muscle fibers by compensatory branching of surviving motor axons, as postulated by van Harreveld ('45), and by Weiss ('46).

Degree of Cell Injury in Relation to Functional State and to Virus Multiplication. It is interesting to speculate on the possible relationship between the degree of cytological injury and of functional impairment. It is probably safe to assume that many neurons quite devoid of Nissl substance are irreversibly injured ("acidophilic necrosis") and non-functional, especially since their nuclei also show severe changes. At the other extreme, it appears that motoneurons showing early mild chromatolysis may still be functionally intact. In several of our preparalytic cases the overwhelming majority of motoneurons supplying an extremity showed mild degrees of chromatolysis in the absence of clinical signs of weakness in the extremity. Of greater interest is the suggestion of our statistical analysis that, whereas neurons showing mild chromatolysis are functional, those showing severe chromatolysis are on the borderline of function and lack of function. Many which are functionless probably recover function during the first month or two. This is not only apparent in the analysis of our paralytic cases, but explains the absence of weakness or very slight weakness in animals inoculated with "mild" strains, yet showing chromatolytic changes in over 90% of motoneurons of the limb segments. In such cases, since virus titers in infected cord may be as high as those in severely paralysed cases, it is probable that considerable virus multiplication in infected neurons need not result in cell death although the host cells undergo reversible chromatolytic changes. The difference between "mild" and "severe" strains appears to reside only in the greater necrotizing effect of the latter.

The progressive diffuse chromatolysis most likely represents an orderly process of regression of motoneuron functions, and since it is the predominant visible cytopathological change in the preparalytic and early paralytic period, when virus concentration in the tissue increases most rapidly (Bodian and Cumberland, '47), it may conceivably be associated intimately with the actual process of virus multiplication. The fact that the Nissl substance is known to be composed at least in part of ribonucleoprotein (Landstrom, et al., '41, Gersh and Bodian, '43) makes this speculation especially attractive.

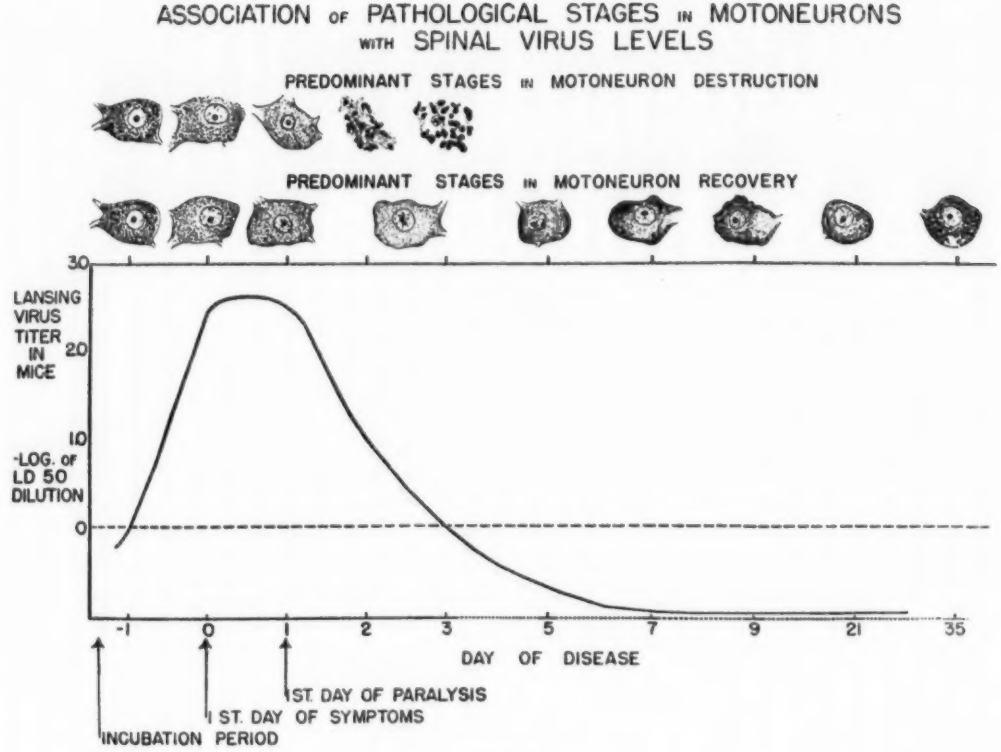
ASSOCIATION OF PATHOLOGICAL STAGES IN MOTONEURONS WITH SPINAL VIRUS LEVELS
PREDOMINANT STAGES IN MOTONEURON DESTRUCTION
PREDOMINANT STAGES IN MOTONEURON RECOVERY
30
20
10
0
LANSING VIRUS TITER IN MICE
-LOG. OF LD 50 DILUTION
-1
0
1
2
3
5
7
9
21
35
DAY OF DISEASE
1ST. DAY OF PARALYSIS
1 ST. DAY OF SYMPTOMS
INCUBATION PERIOD

CHART 5

Nevertheless, the progressive diminution of Nissl substance may also conceivably represent the results of a gradual deterioration of cell metabolism due to the interference of virus multiplication with other cell functions. Chart 5 illustrates the time course of changes in virus concentration, as previously described (Bodian and Cumberland, '47), and the association in time of stages in the cytopathological process which dominate at each period. The fact that the greatest multiplication of virus occurs at the time when the predominant cytological change is that of early diffuse chromatolysis is suggestive. After the peak of virus concentration is achieved, stages of "central" chromatolysis appear and gradually predominate over the "diffuse" chromatolysis characteristic of the earlier period. These stages of central chromatolysis are not only associated with reduction of virus activity, but also with cessation of increase of paralysis, and then with the early recovery from paralysis.

Persistent Perivascular Lesions. The inflammatory changes in acute experimental poliomyelitis have been dealt with exhaustively by Hurst ('29) and by Pette, Demme, and Környey ('32), and nothing new has been added by this study. It is interesting to dwell momentarily on the finding of increasing size of perivascular lymphocytic infiltrations in the subacute and convalescent stages. Such infiltrations sometimes reach massive proportions as late as the second month, often when the interstitial infiltrations no longer contain an appreciable number of these cells. The perivascular "cuffs" are negligible in size, or absent,

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CHART 5. SCHEMATIC REPRESENTATION OF SEQUENCE OF CYTOPATHOLOGICAL STAGES IN MOTONEURONS IN THE COURSE OF DESTRUCTION, AND THOSE CHROMATOLYZED BUT ABLE TO RECOVER.

The approximate time course of changes is shown, with a parallel curve showing the trend of rise and decline of virus concentration in the rhesus spinal cord (from Bodian and Cumberland, '47). Note that peak levels of virus concentration are attained at the time when the predominant stage of cell change in the motoneuron population is that of diffuse cytoplasmic chromatolysis.

The curve is a partly hypothetical one, showing Lansing virus activity in the rhesus spinal cord. The curve up to the third day of paralysis is based on median values of several specimens of infected monkey cord taken at each time period and titrated in mice. The decline beyond the third day of paralysis is shown in a speculative way, and is based on the decreasing probability of obtaining virus in concentrations infective by monkey passage.

in most cases after the sixth month. Warburg's ('31) interpretation of such persistent lesions as a sign of smoldering activity and therefore as an indication for prolonged bed rest must be considered with much reservation. First of all, no evidence that such persistent lesions are associated with virus activity can be brought forward, whereas available evidence indicates that virus activity is negligible after the third week, if it persists that long. Secondly, recrudescence of the disease at such a late date has not been observed in monkeys who are always active to the limit of their capability. Another interpretation of the increase of perivascular lymphocytic infiltrations in the subacute and convalescent stages is suggested by the finding that local antibodies reach a high level in infected regions of the CNS at this time, although relatively low in titer in the serum (Morgan, '47), and by the recent evidence that lymphatic tissue is concerned in antibody formation (McMaster and Hudack, '35; Ehrich and Harris, '42; Dougherty, Chase, and White, '44). It may not be too far-fetched to suppose that the reacting lymphatic tissue exhibits a continuing response in the subacute and convalescent stages to the antigenic stimulus of the acute stage, rather than being an "inflammatory" response to continued virus activity. This suggested significance of the delayed lymphocytic cuffing, however, can only be considered very tentatively until experimental evidence is available.

SUMMARY

1. A description is given of the course of a poliomyelitic infection in the spinal cords of rhesus monkeys killed at intervals from the preparalytic period to the chronic stage.

2. Cytopathological changes in motor nerve cells were studied quantitatively to enable correlations with changes of virus activity on the one hand and changes in clinical motor function on the other.

3. The sequence of cytopathological changes in intracellular structures is considered. Those occurring in the basophilic Nissl substance of the cytoplasm appear to be valid indicators of the functional state of the neuron. Mitochondria and neurofibrils were found to be morphologically intact except in necrotic cells. It was found that axons degenerated between three and four days after cell body destruction, but usually not earlier than the third day. This is the typical time

course of secondary degeneration, and indicates that the primary damage to motoneurons is in the cell body. Nuclear changes were found to occur only after onset of cytoplasmic chromatolysis and are described. The signs of irreversible nerve cell injury are discussed.

4. The general features of the cellular pathological picture are described for stages of the disease from the preparalytic to the convalescent periods.

5. The cytopathological changes in motoneurons were grouped in presumptive stages of regression and recovery and studied with respect to the changing proportions of normal cells, abnormal stages, and destroyed cells in the motoneuron populations supplying single limbs. Limbs of all grades of paralysis were studied, with the average of the order of "moderately severe" paralysis.

6. In paralytic animals it is shown that the process of nerve cell destruction rapidly reaches a peak within the first few days after onset, and shortly thereafter is negligible in relation to the total cell population.

7. In paralytic animals, also, it is shown that as a rule over 90% of all motoneurons in the limb populations are infected during the first few days, as is indicated by abnormal cytopathological changes in the cells. This is true of limbs with mild or transient paralysis as well as those severely paralysed.

8. These changes first involve the Nissl substance, and are progressive in an orderly fashion until changes set in which seriously involve nuclear structure. Short of such irreversible changes, the cytopathological changes in Nissl substance are slowly reversed during the course of 4 to 6 weeks, or less, so that after this time most remaining motoneurons are normal in appearance.

9. Statistical evidence from populations of motoneurons studied during acute and recovery periods indicates conclusively that a large proportion of motoneurons injured to the extent of severe loss of Nissl substance recover their normal appearance. The proportion is greatest in limbs with at least moderate paralysis, but is high in all but the most severely paralysed limbs, in which little function ever returns. In not a single paralytic case between the second and seventh days of paralysis was there found more than 31% of normal cells, even in cell populations supplying limbs with minimal or no apparent paralysis.

10. The relation between damage and recovery of the motor nerve cell population to motor paralysis and recovery is considered. It appears that the degree of motoneuron destruction alone in a limb motoneuron population can account for most of the weakness observed in the corresponding limb in the period between 21 days and 3 months after onset of paralysis. On the average, limbs graded as normal in this period had lost about $\frac{1}{3}$ of their motoneurons, and those graded as completely paralysed still retained about 10% of the motoneuron population.

11. The degree of weakness in the acute stage was greater than could be accounted for by nerve cell destruction alone, but evidence suggests that the discrepancy may be related to the absence of function in severely chromatolysed motoneurons which apparently later recover their function. There is evidence that suggests that neurons showing mild degrees of chromatolysis are still functional in that state.

12. The distribution of neuron lesions was almost universal in the nerve cell populations of all four limbs in paralysed animals and in most animals with non-paralytic poliomyelitis. This was also true of animals infected with "mild" strains and showing very slight weakness. No consistent pattern of cell invasion was found, but there was a suggestion that cells on the periphery of the anterior columns of limb enlargements had a better chance for survival. Some of the factors which may be involved in determining the pattern of paralysis are discussed.

13. Evidence is presented which indicates that highest concentrations of virus are achieved in the anterior horn when the predominant pathological change in the motoneuron population is that of diffuse cytoplasmic chromatolysis of Nissl substance.

14. Perivascular lymphatic accumulations, occasionally with germinal centers, persist for months after acute neuronal changes, acute inflammatory changes, or evidence of virus activity can be found. It is suggested that such persisting lymphatic tissue may be associated with the occurrence of local antibody.

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Explanation of Illustrations

Except where otherwise noted, sections were prepared at 15 micra and stained with gallocyanin.

(Photographs were prepared by Mr. Chester F. Reather)

Plate 1. Regressive Stages in Spinal Cord Motoneurons in Poliomyelitis. Rhesus B12, First Day of Paralysis, × 480.

Fig. 1. *Normal anterior horn cell.* Note massive Nissl bodies in cytoplasm, central position of nucleus, large nucleolus, and dispersed chromatin.

Figs. 2, 3, 4. Early regressive stages. Note diffuse decrease in size of Nissl bodies (chromatolysis), and nucleus essentially normal.

Fig. 5. Severe diffuse chromatolysis, with only a few small masses of Nissl substance in cell periphery. Note clumping of chromatin in nucleus.

Fig. 6. Complete dissolution of Nissl bodies in cytoplasm, which is slightly basophilic in staining. Nucleus slightly shrunken and containing a small eosinophilic inclusion body.

Fig. 7. Cell similar to that in fig. 6, with further shrinkage of nucleus. Note infiltrating "polyblasts" surrounding the nerve cell.

Fig. 8. Completely chromatolytic cell with severe diffuse basophilia of cytoplasm and of shrunken, distorted nucleus.

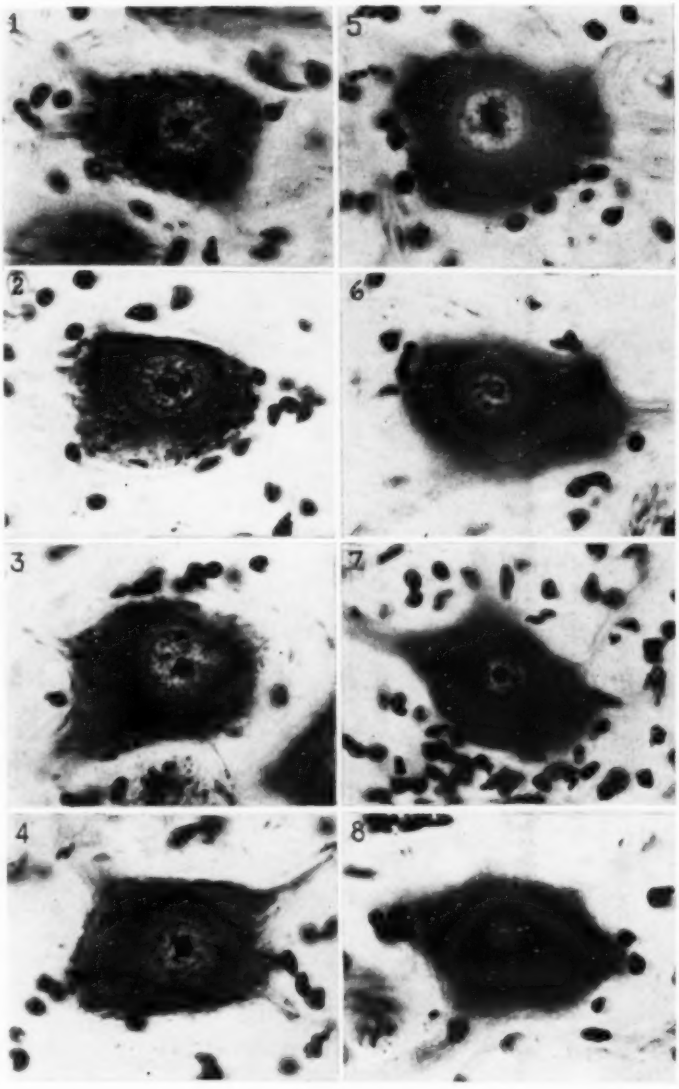
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PLATE 2. Stages of degeneration of motoneurons during first day of paralysis, showing destruction by phagocytosis (fig. 1 to 4), by cytolysis (figs. 5 and 6), and by vacuolation with absorption (figs. 7 and 8). Figs. 1, 2, 3, 5, 7, and 8 from rhesus B12. Figs. 4 and 6 from rhesus A696. × 480.

FIG. 1. Intensely hyperchromatic cytoplasm and shriveled nucleus. Such basophilic cells are more commonly phagocytized than those which become acidophilic. Note blood capillary enclosed by cytoplasm of cell.

FIG. 2. Shrunken, hyperchromatic cell, with granular and vacuolated cytoplasm and pyknotic nucleus. Beginning neuronophagia by surrounding leucocytes and macrophages.

FIG. 3. Active neuronophagia of necrotic cell.

FIG. 4. Macrophages and glia cells at site of a completely phagocytized cell.

FIG. 5. Complete chromatolysis, with no trace of basophilia in cytoplasm. Such cells appear to be on the verge of "acidophilic necrosis", or the cytolysis and vacuolation shown in figs. 6 to 8.

FIG. 6. Cytolysis of cell with shrunken nucleus showing a beaded, basophilic border. After complete cytolysis, a punched out, fluid-filled cavity is left ("falling out"), which is reduced in a short time by shrinkage and the infiltration of macrophages, etc.

FIGS. 7 AND 8. Cells in final stages of vacuolation, with surrounding and invading phagocytic cells.

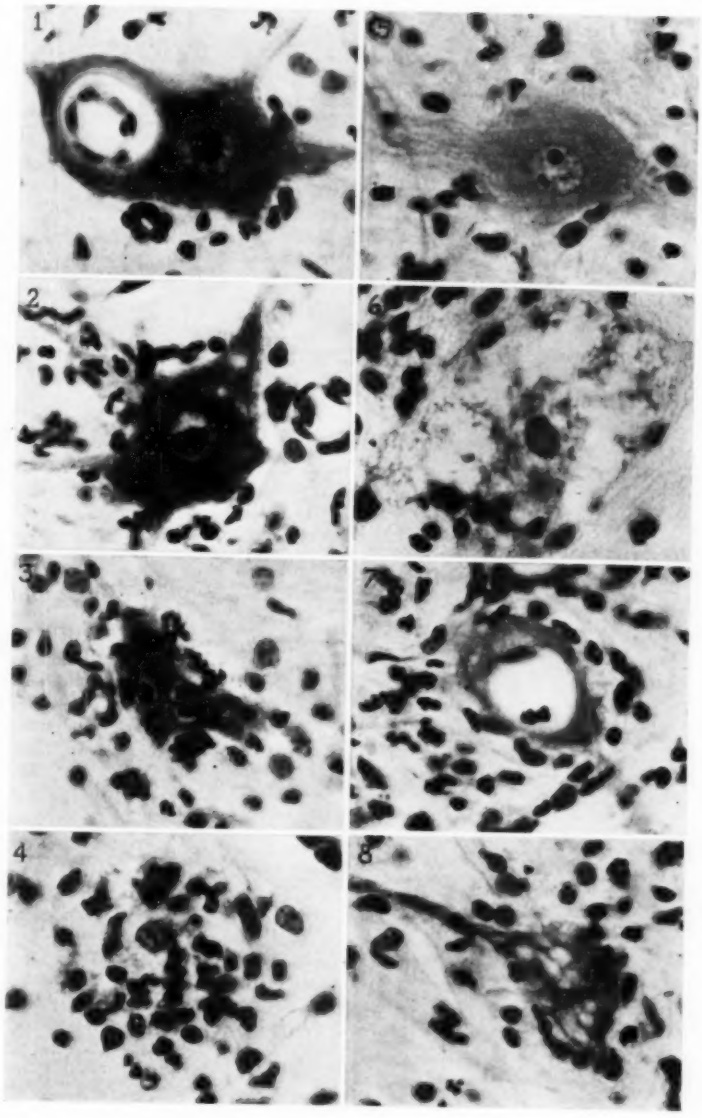
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PLATE 3. DEGENERATION OF MITOCHONDRIA IN POLIOMYELITIS

FIG. 1. Anterior horn cell in stage of severe poliomyelitic chromatolysis, with eccentric nucleus (n) containing several inclusion bodies. Note numerous granular and small rod-like mitochondria in cytoplasm (c). The pale larger masses at the edges of the cytoplasm are remaining Nissl bodies. Rhesus B916, first day of paralysis. Regaud, Iron-haematoxylin, 7μ, $\times$ 1300.

FIG. 2. Anterior horn cell in same section as fig. 1, showing necrotic changes in nucleus (n) and cytoplasm (vacuolation, granular degeneration). No mitochondria or Nissl bodies visible in cytoplasm (c).

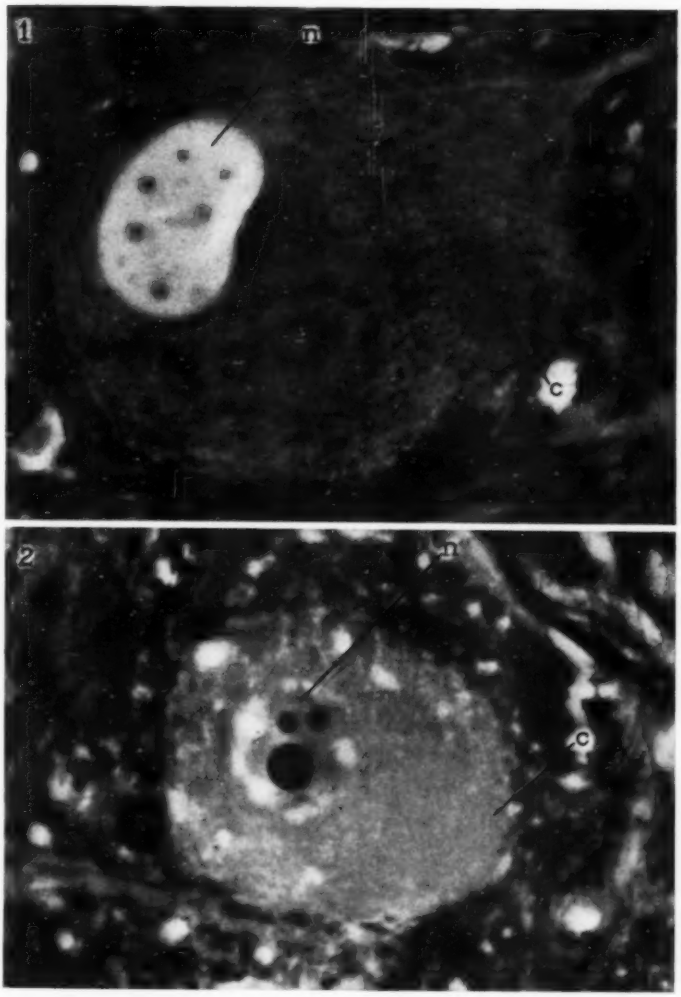
1
n
c
2
n
c

PLATE 4. DEGENERATION OF MOTOR NERVE FIBERS IN POLIOMYELITIS.
PROTARGOL STAIN, 15μ, $\times$ 560

FIG. 1. Normal anterior spinal root of rhesus monkey, showing large and small axons of fairly uniform caliber. Rhesus C888.

FIG. 2. Emerging anterior root fiber in white matter of spinal cord of rhesus monkey on third day of paralysis. Note swelling and vacuolation of axon (ax). This represents the earliest degenerative change seen in our series. Rhesus C947.

FIG. 3. Sixth lumbar anterior spinal root of monkey with complete paralysis of left leg, showing severe degenerative changes of almost all axons in the root. Fourth day of paralysis. Rhesus C944.

FIG. 4. Sixth lumbar anterior spinal root of monkey with complete paralysis of left leg, showing complete degeneration and absorption of all axons, with replacement by neurilemmal and fibrous tissue, 57 days after onset of paralysis. Rhesus C437.

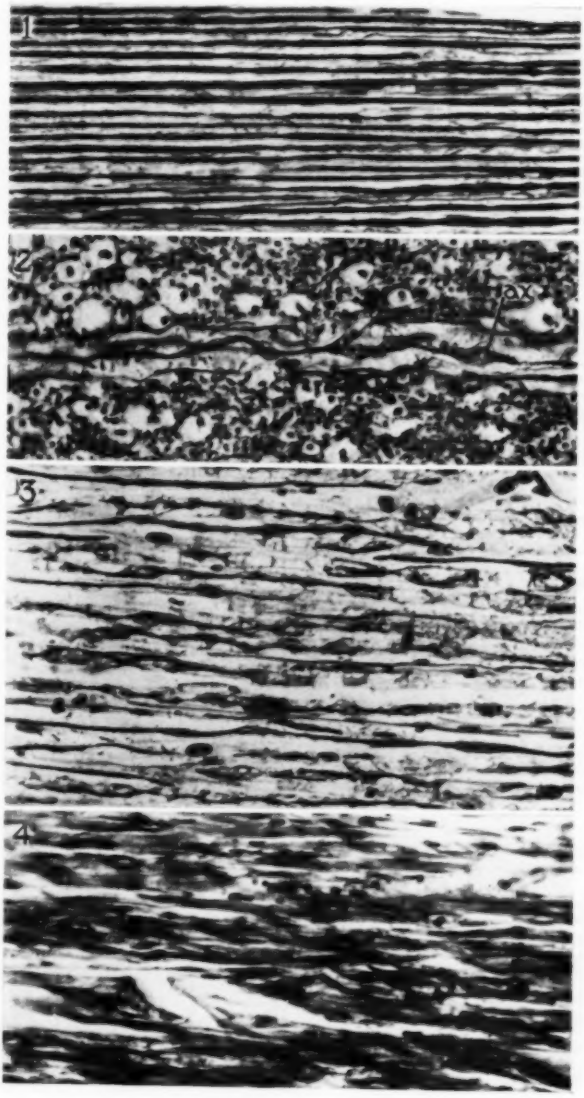
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PLATE 5. Samples of the infrequently occurring motor nerve cells which persist after the acute period, although they show changes of a presumably irreversible nature ("necrotic").

FIGS. 1 to 4, chimpanzee A434, 7 days after onset of paralysis; Fig. 5, rhesus B32, 9 days; Fig. 6, rhesus B35, 21 days. × 480.

FIG. 1. Motoneuron with eosinophilic, highly refractile cytoplasm, with shrunken and distorted cell and nuclear membranes.

FIGS. 2 AND 3. Similar cells, but with strongly basophilic nuclei.

FIG. 4. Markedly shrunken cell, with strongly basophilic masses in nucleus and cytoplasm, surrounded by macrophages. Normal internal structure is not visible.

FIG. 5. Cell with distended nucleus, and glassy, eosinophilic cytoplasm, probably irreversibly damaged.

FIG. 6. Motoneuron with glassy cytoplasm, and rim of basophilic substance, showing nucleus apparently verging on extrusion from cytoplasm. (See also Plate 10, fig. 1).

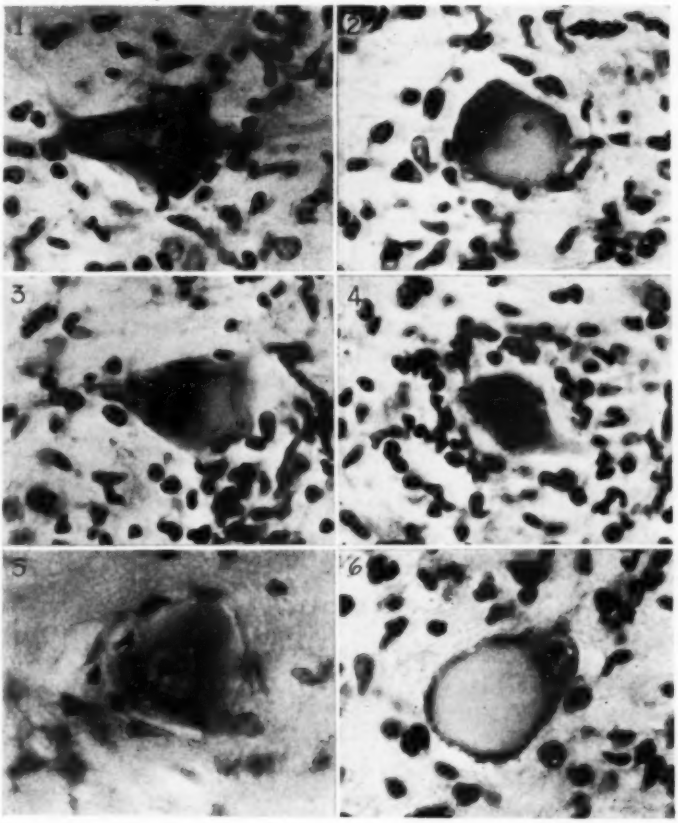
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PLATE 6. EARLY PREPARALYTIC POLIOMYELITIS. RHESUS A935

FIG. 1. Section of cord at fourth lumbar level, showing characteristic fully developed inflammatory lesions. cc—central canal. pr—perivascular infiltration around vessels in anterior fissure. × 60.

FIG. 2. Motoneurons also shown in box in fig. 1, at higher magnification. Note early diffuse chromatolysis. × 250.

FIGS. 3–5. Sections at distance of 1½ mm. from section shown in fig. 1. These sections, and adjacent ones, had no infiltrative cells in anterior horns. Only the section shown in fig. 5 had any infiltration in the entire section, and this was around the anterior spinal vessels. The diffuse chromatolysis seen in the cells at the left was seen in these cells alone in their respective sections, and in all three cases was in the ventrolateral cell column. × 250.

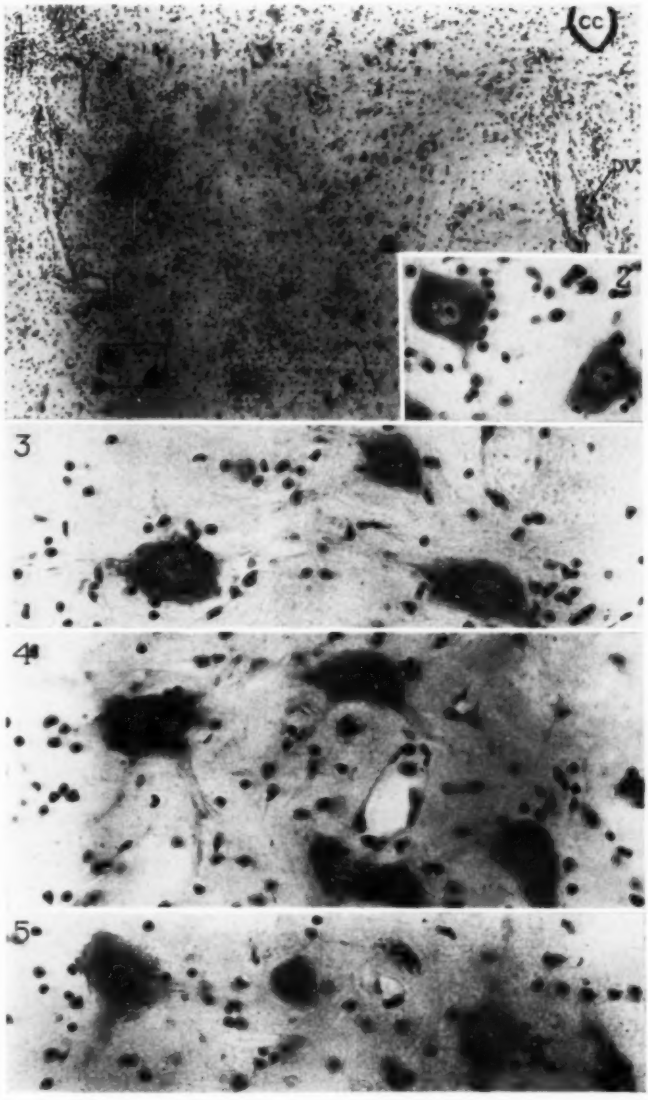
CC
DV
2
3
4
5

PLATE 7. Rhesus B338. Fifth day after onset of paralysis. Series of cells showing the transition from regressive changes (diffuse chromatolysis) to recovery stages (central chromatolysis). × 480.

FIGS. 1 AND 2. Motoneurons showing the diffuse cytoplasmic chromatolysis characteristic of the acute stage.

FIGS. 3 AND 4. Motoneurons showing a tendency for massing of Nissl substance near cell and nuclear membranes, and diffuse cytoplasmic basophilia. Such cells are apparently transitional between those of figs. 1 and 2, and 5 and 6.

FIGS. 5 AND 6. Motoneurons showing central chromatolysis, with well-stained Nissl bodies and absence of diffuse basophilia. Such cells are characteristic of the entire recovery period.

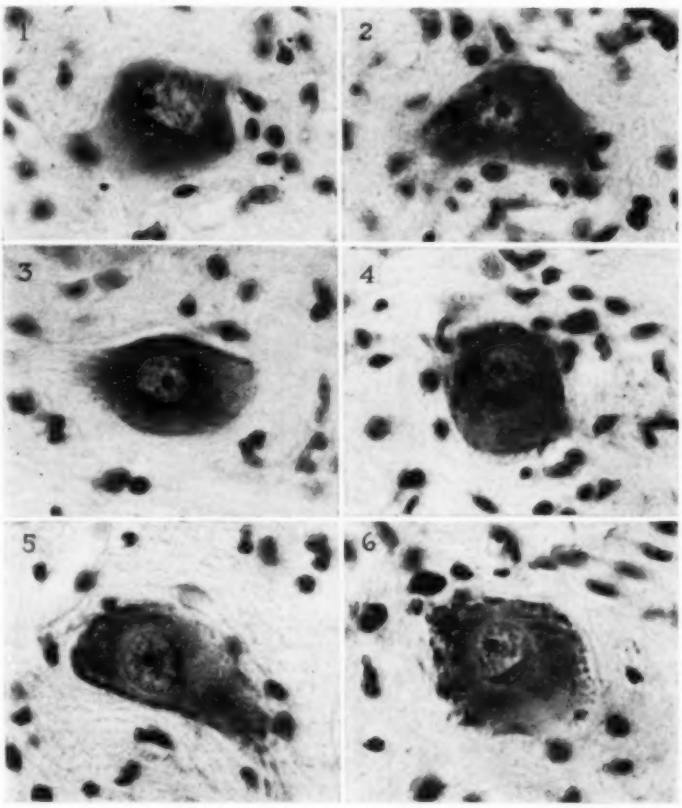
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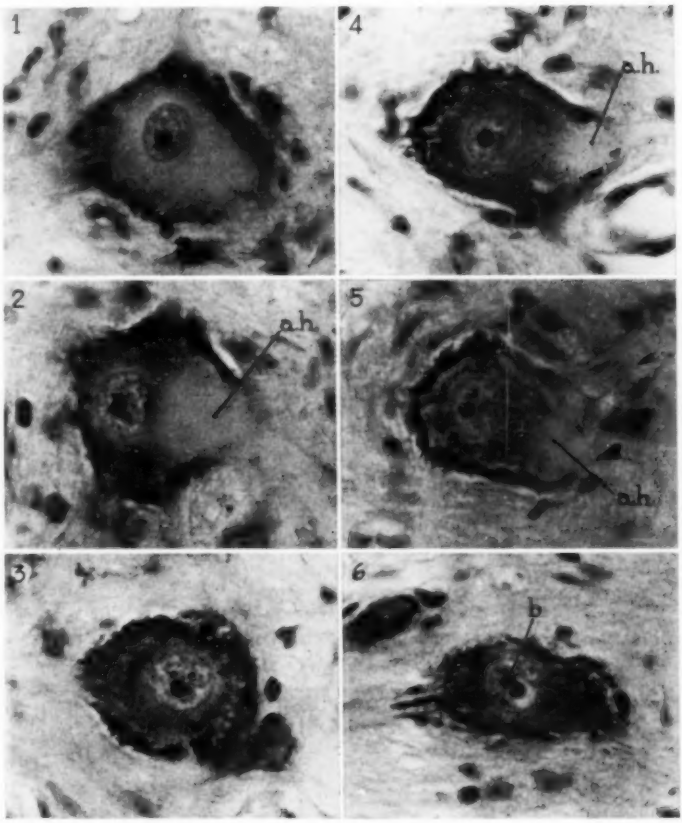
PLATE 8. RHESUS B32. NINTH DAY AFTER ONSET OF PARALYSIS. × 480.

FIG. 1. Severe central chromatolysis, with normal-appearing nucleus, and accumulation of heavy masses of Nissl substance near cell membrane. Regeneration of Nissl substance may or may not occur near the nuclear membrane.

FIGS. 2 TO 5. Similar cells but with small Nissl bodies in central area. This appearance suggests regeneration of Nissl bodies from the periphery inwards, with the area around the axon hillock (ah) last to show recovery (figs. 2, 4, and 5).

FIG. 6. Motoneuron of essentially normal appearance, except for presence of acidophilic inclusion body (b) in nucleus. In the acute stage such inclusion bodies are seen only in cells with severe chromatolysis, suggesting almost complete recovery of such a cell.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a.h.
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a.h.
3
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b

PLATE 9. INCOMPLETELY RECOVERED MOTONEURONS INFREQUENTLY SEEN AFTER THE THIRD WEEK. × 480.

FIGS. 1 AND 2. Rhesus B35, 21 days after onset of paralysis. Central chromatolysis similar to that seen in Plate 8, with acidophilic inclusion body (b) in fig. 2.

FIGS. 3 TO 6. Rhesus B190, 35 days after onset of paralysis. Motoneurons in late stages of recovery, but showing various signs of previous injury; fig. 3, incomplete recovery of Nissl bodies in central area; fig. 4, normal cell, except for acidophilic intranuclear inclusion (b); fig. 5, cell with almost recovered Nissl bodies, but with bizarre basophilic intranuclear inclusion body (a), representing agglomerated nucleolus and surrounding chromatin; fig. 6, otherwise normal cell with similar intranuclear formation.

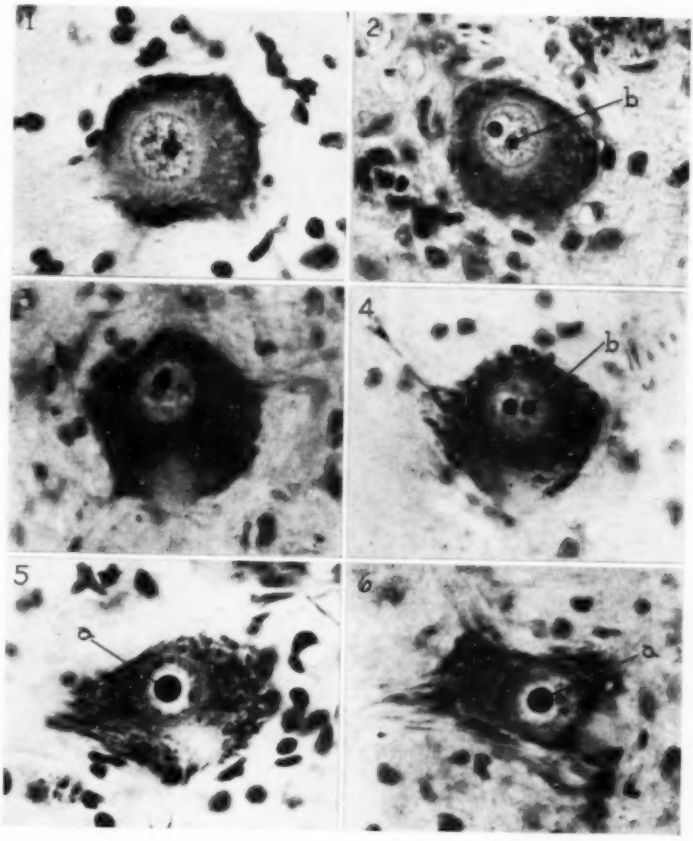
1
2
b
3
4
b
5
a
6
a

PLATE 10. Rhesus B935, 7 days after onset of paralysis, showing the changing character of reacting non-neuronal cells after the acute stage. Regaud, haematoxylin-eosin-azure II, 7 micra, × 560.

FIG. 1. Note absence of polymorphonuclear leucocytes, "rod" cells, or of the irregular "polyblasts" of the acute stage. Spindle-shaped cells (h), probably histiocytes or microglia, dominate the scene, which also contains glia cells, and a few stray lymphocytes. The moribund motoneuron in the center appears to be undergoing extrusion of its nucleus (n).

FIG. 2. Same section as fig. 1. Note the mixed character of cells in the perivascular zone. In this zone in the acute stage the cells are largely lymphocytic in type. Histiocytes and macrophages are numerous, as well as lymphocytes and a few cells of the "stem-cell" type, with cytoplasmic basophilia. Some of the macrophages contain engulfed lymphocytes at this stage (m). The neuron at upper left is quite abnormal in appearance and contains a basophilic intranuclear inclusion of the type shown in Plate 9, figs. 5 and 6.

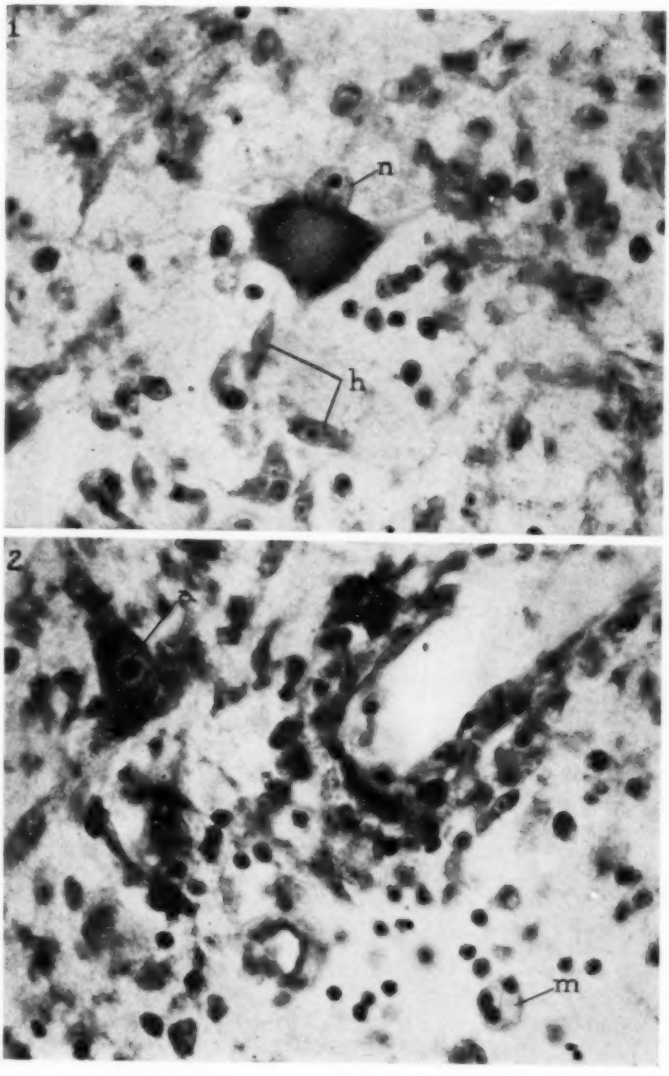
1
n
h
2
m

PLATE 11. Rhesus B190, 35 days after onset of paralysis, showing the second stage of lymphocytic perivascular infiltration in the early convalescent period, when lymphocytes are largely absent from the rest of the tissue.

FIG. 1. Massive perivascular lymphocytic infiltrations, containing "germinal" centers (c). × 65.

FIG. 2. Same at higher magnification showing area at lower left. "Germinal" center contains stem cells and mitotic figures (m). × 270.

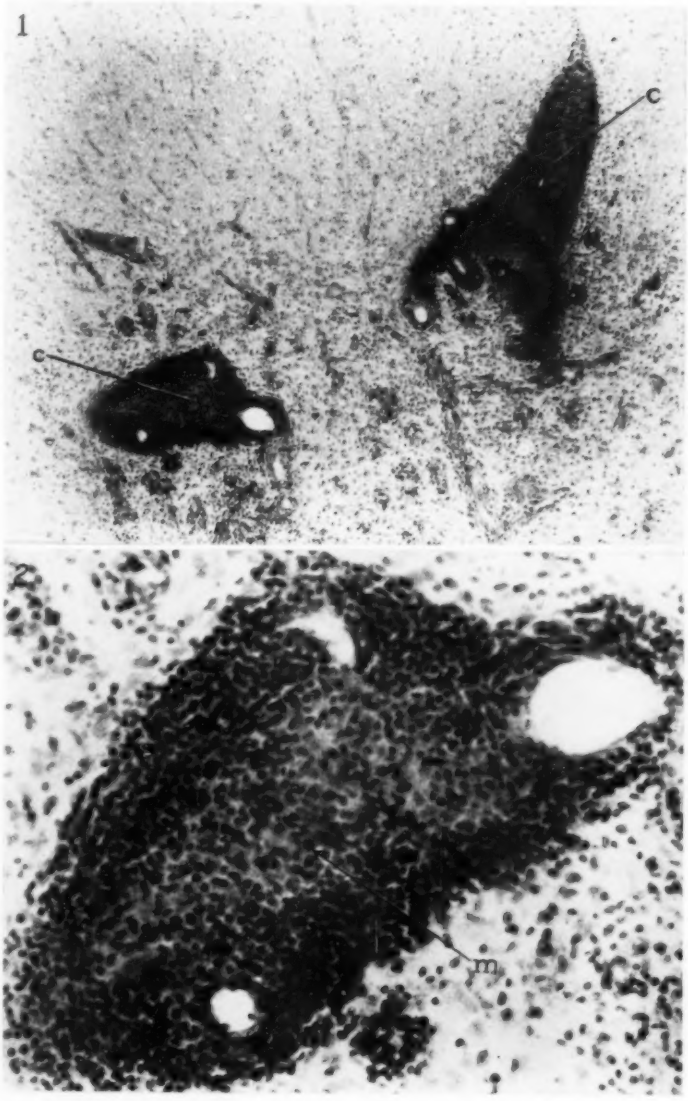
1
c
c
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PLATE 12. Normal rhesus lumbar cord, to show the sharp borders of the area containing the motoneurons of this segment of spinal cord. × 20.

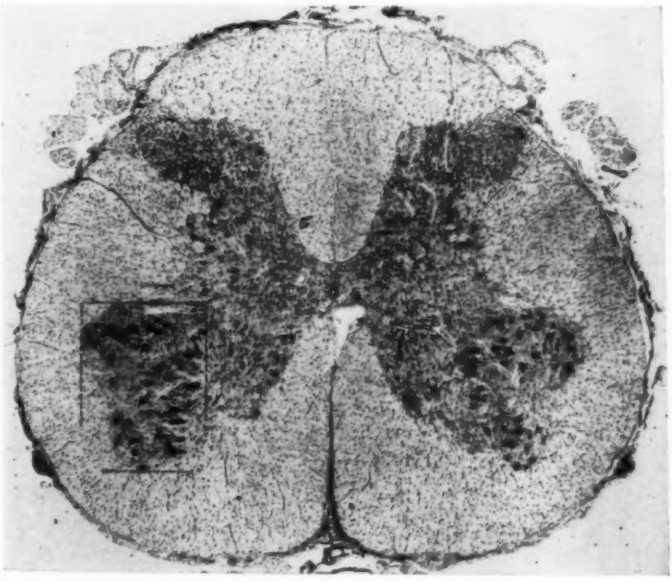

PLATE 13. The principal morphological stages of injured and recovering motoneurons, during the first week of the disease. × 250.

FIG. 1. Rhesus B12, preparalytic period. Note the diffuse character of chromatolysis in the three motoneurons below. The one at upper right is essentially normal.

FIG. 2. Rhesus A794, third day after onset of paralysis. Transitional stage, with motoneurons showing "new" granular Nissl bodies, and accumulation of Nissl substance near cell and nuclear membranes.

FIG. 3. Rhesus B338, fifth day after onset of paralysis. Early stage of central chromatolysis, with definite membrane accumulation of Nissl substance, and pale central zone of cytoplasm.

FIG. 4. Rhesus B935, seventh day after onset of paralysis. Typical central chromatolysis with "filling-in" of central cytoplasmic area by "new" Nissl bodies.

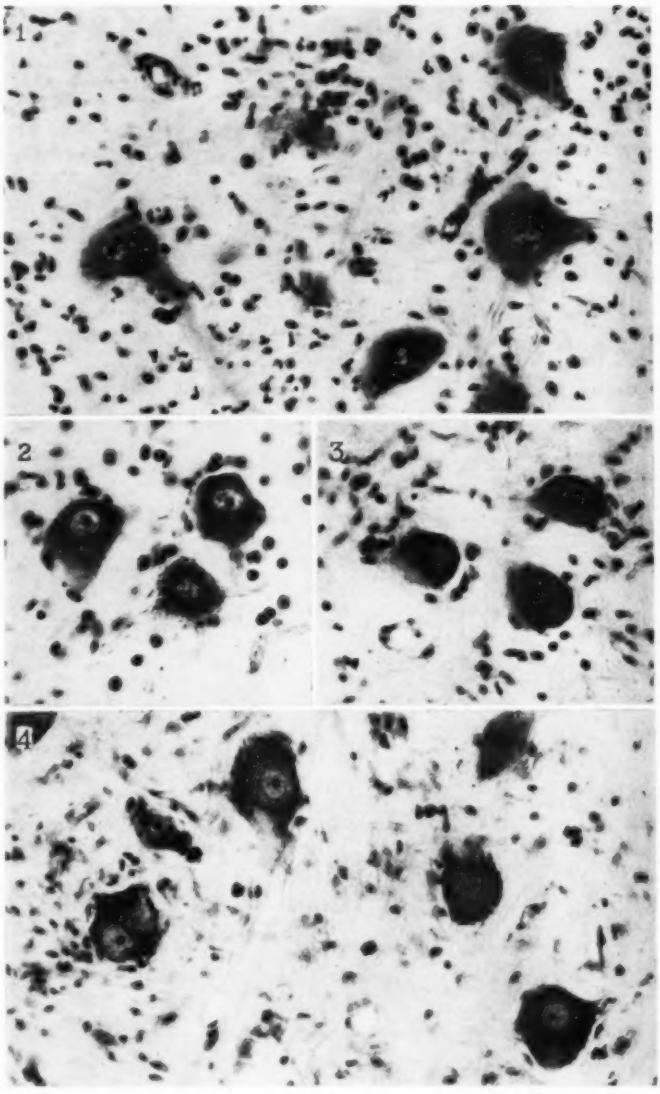
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PLATE 14. Sections through lumbar cord of rhesus C59, inoculated with mild Frederick strain, with transient paralysis after 17 days, and killed 4 days later. Described on page 58.

FIG. 1. Right anterior horn. Note that all motoneurons show chromatolysis, although there is little inflammatory reaction. × 125.

FIG. 2. Left anterior horn, same section, showing neuron loss, as well as chromatolysis of remaining cells. × 80.

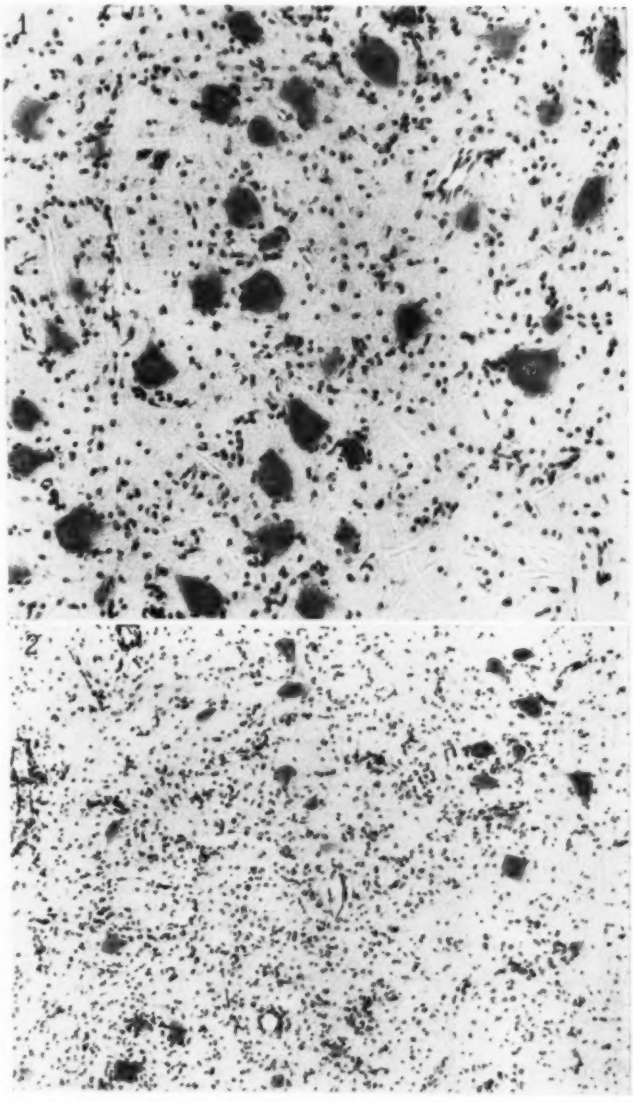
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PLATE 15. CHIMPANZEE A434, SEVENTH DAY AFTER ONSET OF PARALYSIS

FIGS. 1 TO 6 show a group of cells arranged in the sequence of increasing recovery from the chromatolysis of the acute stage. Note the similarity to cells of rhesus monkey B32, shown in Plate 8. × 480.

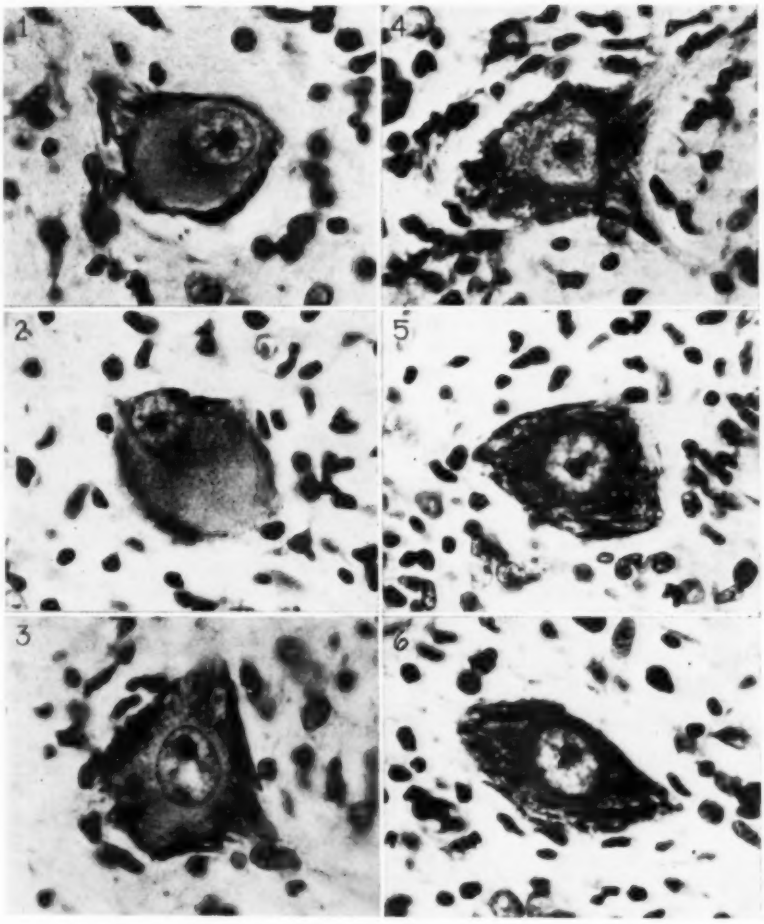
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PLATE 16. Rhesus C479, showing the acute stage of non-paralytic Western equine encephalomyelitis in the cervical spinal cord. × 270.

FIG. 1. Group of anterior horn cells showing either normal appearance or severe diffuse cytoplasmic chromatolysis. Small acidophilic intranuclear inclusions are visible in the chromatolytic cells.

FIG. 2. Neighboring section, showing characteristic inflammatory focus, with the same cell types seen in poliomyelitis. A single necrotic motoneuron is seen (x), but most motoneurons show no changes, in contrast with poliomyelitis cases.

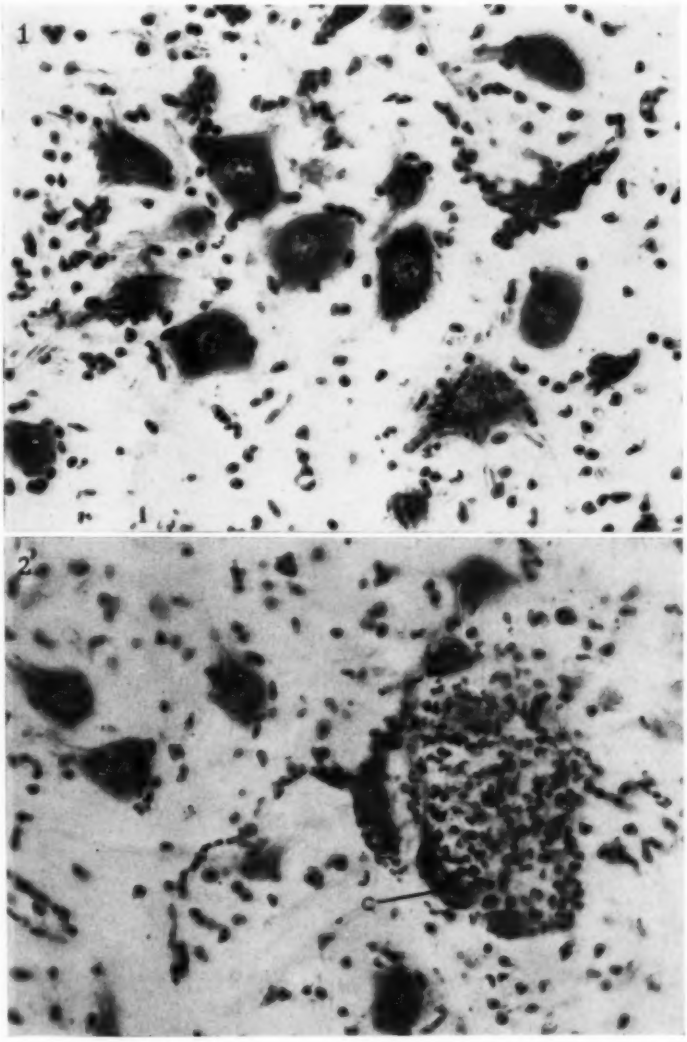
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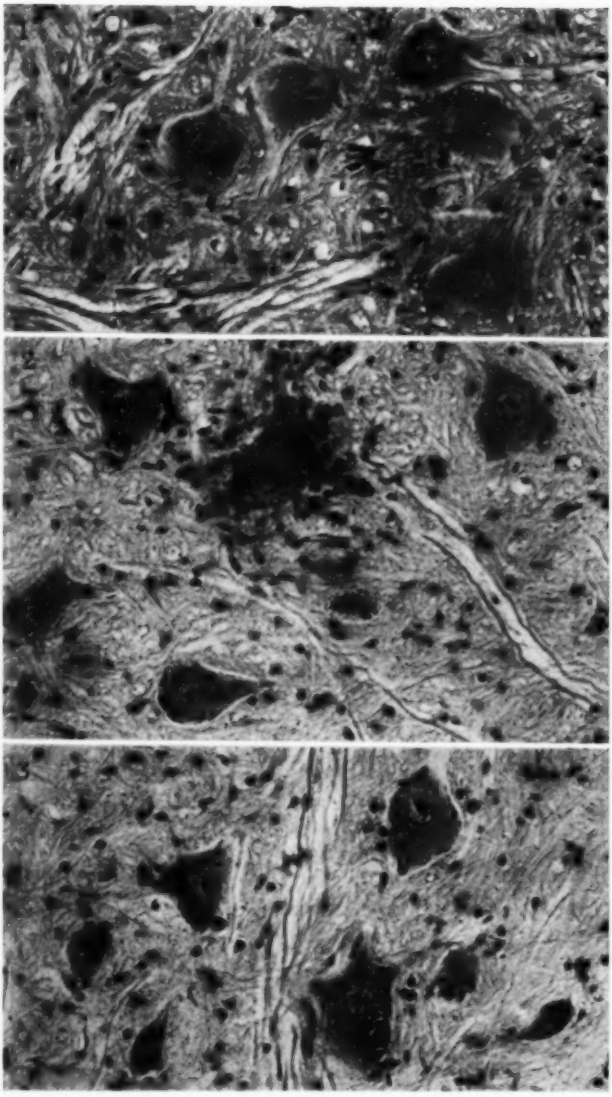
PLATE 17. Three stages showing the injury and recovery of motoneurons following non-paralytic infection with neurotropic yellow fever virus (17D strain). Toluidin blue, × 250.

FIG. 1. Rhesus C481, two days after onset of disease. Note the diffuse chromatolysis of the two lower motoneurons, in the absence of inflammatory reaction. Nuclei are normal.

FIG. 2. Rhesus C480, five days after onset of disease. Note the beginning membrane accumulation of Nissl bodies in motoneuron below and at upper right. Nerve cell above in center shows intense basophilia of nucleus and cytoplasm, although morphologically almost intact. Such cells were not frequent in occurrence.

FIG. 3. Rhesus C482, eleven days after onset of disease. Two large motoneurons at right show typical central chromatolysis, similar to that seen in poliomyelitis at this period in the disease. Other motoneurons in the section resemble most motoneurons in the spinal cord in being normal in appearance.



PROCEEDINGS OF THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

Held in Hurd Memorial Hall, April 12, 1948

ELECTION OF OFFICERS FOR THE COMING YEAR

Dr. A. McGehee Harvey was elected president and Dr. Richard H. Follis was elected secretary-treasurer for the year 1948–49.

THE RELATIONSHIP BETWEEN INFLUENZA AND ACUTE BACTERIAL PNEUMONIA

Conflicting Interpretations of Laboratory and Epidemiological Evidence. Dr. Alexander D. Langmuir. (Department of Epidemiology, Johns Hopkins University School of Hygiene and Public Health.)

During the 1928 pandemic of influenza, bacterial infections of the lung were known to have been unusually frequent and to have contributed substantially to the excessive mortality that was recorded. During the eighteen epidemics of mild influenza that have occurred in the United States since the pandemic, the existence of a definite relation between the virus infection and a complicating acute bacterial pneumonia has been less apparent.

Although each of these eighteen epidemics has been accompanied by a measurable excess mortality from pneumonia, such statistical evidence does not determine whether these excess pneumonia deaths resulted from bacterial invasion of the lungs or merely from extensive virus infections. Laboratory studies have not clarified the question completely. The rarity of bacterial complications during localized outbreaks of influenza A and B has been emphasized on repeated occasions. Studies of patients with acute bacterial pneumonia have yielded variable results. Although relation between influenza and acute staphylococcal pneumonia is now well established, evidence of a similar relation with pneumococcal pneumonia is very limited.

An epidemiological study of the records of the pneumonia service at Bellevue Hospital, New York, is now in progress in collaboration with Dr. Norman Plummer. These records comprise case histories of patients with pneumococcal pneumonia admitted between October 1920 and June 1932. During this period, six epidemics of influenza occurred in New York City. During four of these epidemics, the weekly incidence of admissions for acute bacterial pneumonia increased sharply by 50 to 100 per cent or more. In the other two epidemics slight but less definite increases were observed.

This epidemiological evidence indicates that during certain epidemics of influenza as much as 50 per cent or more of the cases of pneumococcal pneumonia may represent concurrent secondary bacterial complications of primary virus infections.

Elucidation of this mechanism is one logical approach toward a better understanding of pandemic influenza.

Demonstration of Influenza Virus Complicating Pneumococcal Pneumonia, 1946–1947. DR. THOMAS G. WARD. (Department of Bacteriology, Johns Hopkins University School of Hygiene and Public Health.)

Evidence presented herein suggests that influenza virus is one of the causative agents in cases of pneumonia previously considered primarily bacterial in origin. Sputum was obtained from 69 cases of bacterial pneumonia and studied for influenza virus by the chick embryo technique. Of 33 cases occurring during non-influenza periods one yielded an influenza B virus. Of 36 cases occurring during the period when influenza A was prevalent in Baltimore, 13 yielded influenza virus which were serologically similar to influenza A strains isolated from clinical cases of influenza occurring at the samt time. Acute and convalescent blood specimens were secured on 53 of these same cases, and a third specimen was obtained about five months later on 25. Four cases showed evidence of positive hemagglutination-inhibition antibody response but failed to yield virus. Thus a total of 17 of 36 cases (47%) of bacterial pneumonia occurring during an influenza A epidemic gave evidence, by virus isolation or serologic techniques, of the presence of influenza virus associated with bacterial pneumonia.

In addition, a total of 87 specimens of lung secured at autopsy were studied. Two of nine cases whose death was attributed primarily to pneumonia yielded influenza virus. Two of 28 cases whose primary cause of death was complicated by pneumonia also yielded influenza virus. No virus was secured from the remaining 50 cases which presented no evidence of pneumonia. Twenty-eight of these specimens were secured during the influenza epidemic in Baltimore, and none of these yielded virus.

To summarize, these studies demonstrate that influenza virus probably plays an etiological role in the production of bacterial pneumonia in man.

The Clinical Features of Pneumococcal Pneumonia Complicated by Influenza Virus Infection. DR. THOMAS E. VAN METRE. (Department of Medicine, The Johns Hopkins Hospital).

The preceding papers showed that influenza might coexist with bacterial pneumonia. Does influenza modify the course of concurrent bacterial pneumonia? The clinical records of 49 non-fatal cases of pneumococcus pneumonia that were studied for the presence of influenza by Dr. Ward have been analyzed. In 18 of these cases influenza was present by the criteria previously discussed. The 18 cases with influenza showed no significant difference from the 31 cases without influenza beyond a higher incidence of symptoms of influenza preceding or concurrent with the onset of pneumonia. These findings concur with those of Finland on non-fatal cases of pneumococcic pneumonia complicated by influenza.

The clinical and autopsy records on two fatal cases of pneumonia in which influenza virus was isolated from the lungs were presented. The first patient was a 7-month-old, white, male infant who died in four days of a progressive tracheobroncho-pneumonitis. Type XI pneumococcus was present in the sputum, but there was no response to penicillin and sulfadiazine. At autopsy, a necrotising tracheitis and an interstitial bronchitis and pneumonitis were found, the picture of pure virus infection. No bacteria could be stained or cultured. Influenza B virus was isolated from the lung. This case was interpreted as one in which death was due to influenza virus infection.

The second patient was a 25 year old negress who died in 32 hours of a lobar pneumonia involving RML, RLL, LLL, LUL, despite the fact that penicillin and sulfadiazine were started 12 hours after her first symptom. Type II pneumococcus was demonstrated in lung and nasopharynx. Influenza A virus was isolated from the lungs. At autopsy a typical early pneumococcal pneumonia involving all but the lung apices was found. A very few gram-positive, encapsulated diplococci could be stained in the alveoli. There were no lesions typical of virus infection. Moderate mitral stenosis was also found. The presence of influenza A suggested that the virus might have been responsible for the fulminating course and the absence of response to adequate specific therapy for the pneumococcus.

Dr. William B. VandeGrift: What was shown by bacterial stains of the lung in the second case?

Dr. Thomas E. Van Metre: Bacterial stains showed almost no evidence of bacteria. Only three or four gram-positive diplococci could be found in an entire section. Culture of the lungs revealed a few colonies of type II pneumococci.

Dr. VandeGrift: In the second case were the lung lesions typical of virus or pneumococcal pneumonia?

Dr. Van Metre: In the second case the lung lesions were typical of pneumococcal pneumonia.

Dr. VandeGrift: I disagree with the diagnosis of pneumococcal pneumonia. The description and illustrations indicate a diagnosis of acute virus pneumonia. They are quite similar to the changes in a case of mine who died two days after an exacerbation with a spread to the left upper lobe. Microscopic examination showed edema and infiltration with neutrophiles of the alveolar septae, fibrin lining of the alveoli, and a variable amount of purulent exudate in the alveoli which contained, in addition, edema fluid, macrophages and desquamated alveolar epithelium. In the entire right lung and left lower lobe were the usual findings of acute bronchitis, bronchiolitis and infiltration of the peribronchial tissue with plasma cells, lymphocytes and occasional eosinophiles.

Dr. Van Metre: Well, all that I can say is that perhaps you have one of the cases that we are looking for. Maybe the findings in your case and in the case under discussion are all that would be expected in an early case of influenza virus and pneumococcal pneumonia. However, on review of sections of my case with people more experienced that I—and sections were taken from all lobes—there was nothing

that could be seen in that pneumonia that could not be seen in pneumococcal infections. We believe from the clinical and virological standpoint that the virus may well have played an important role. I am delighted to hear that you have had a similar case.

Dr. Arnold R. Rich: I think the studies that the speakers have carried on are immensely important because, from the pathological side, we have difficulty in knowing just what to think about the relation of influenza to pneumonia in non-pandemic times. Of course, during the pandemic influenza, the effects of the virus were visible and were seen by everybody. It was well recognized that the virus produced a mononuclear infiltration of the bronchial walls, and that the bacteria produced a purulent exudate in the bronchial lumen and in the alveoli. The studies of Shope have, of course, shown that very beautifully in swine influenza, too; and we are all familiar with the effect of another virus, that of measles, which also renders the body susceptible to bacterial pneumonia. There, too, the walls of the bronchioles and bronchi are infiltrated with mononuclear cells, and we recognize that as characteristic of the virus, with the superimposed bacteria producing the purulent exudate. Now the curious thing is that in non-pandemic influenza periods, even though influenza may be prevalent in the population, we very rarely see this bronchial infiltration with mononuclear cells in adults who die of pneumonia. It is a rarity. I think that the question arises right away as to whether the influenza viruses that are present in non-pandemic times are different from the pandemic virus in that they do not call forth the mononuclear infiltration of the bronchial walls, or whether the pneumonia that we see outside of pandemic influenza periods is pneumonia that is not associated with influenza.

In relation to Dr. Langmuir's studies, since influenza flourishes in periods in which other respiratory and bacterial infections are common, I would like to ask whether it would be possible to set up curves, similar to the one showing a parallelism betwen influenza and pneumonia, for other infections and pneumonia. Could the influenza-pneumonia correlation have been coincidence?

I would like to ask Dr. Ward how often the influenza virus would be found, during an influenza epidemic, in the sputum of individuals who didn't have influenza. The possibility of terminal aspiration of sputum must be borne in mind in relation to the isolation of influenza virus from the lungs at autopsy. It is very common that a person who is dying aspirates his sputum. When influenza is widespread in the population would it not be possible to find, in patients without influenza, influenza virus in the lung that had been aspirated terminally? I think that is an important point.

Some of you may remember that last year Dr. Ward, Dr. Van Metre, and the pathology department, at a Monday Clinical Pathological Conference, went over some of these cases, and we were not able to reconcile, as well as we would like to have done, the pathological picture with the findings of the virus studies. I repeat that the thing that bothers us is that in patients who died of pneumonia following the pandemic influenza mononuclear infiltration of the bronchial walls was so

widespread and so obvious, and it is so obvious in relation to measles, which also predisposes to pneumonia; but in epidemic influenza periods, adults dying from pneumonia do not show it, and we would like to know why. You have seen that in the adult case presented by Dr. Van Metre. Influenza virus was isolated in high titer, but the bronchioles did not show the mononuclear infiltration. I think, from a study of the lesions in that case, that there is nothing there that one wouldn't see in an early pneumococcal pneumonia uncomplicated by influenza. Continued studies such as these will, I think, lead us to understand what these discrepancies really mean.

Dr. Thomas B. Turner: I would like to make one or two comments which are somewhat tangential to the main question under consideration. First, I think it is obvious that, using these biological tests in the isolation of influenza virus, it is not possible to isolate virus in 100 per cent of the cases in which it is present. Second, two things of considerable interest were referred to in passing. Some of these isolations were made at inter-epidemic periods. We have all wondered what happens to influenza virus between epidemics; it is perfectly clear that the virus is present in the community in occasional cases in these inter-epidemic periods and that is perhaps the way it is kept alive. Further, a very important aspect is the great variation in antigenicity of these different strains. It is quite possible that the strains now included in influenza are not by any means the best immunizing strains to use. Perhaps after testing large numbers of strains one or two can be found which will be much more protective than the vaccine now available.

Dr. Alexander D. Langmuir: Dr. Rich has really put his finger on the toughest problem. Certainly one simple explanation for the increase in pneumonia with each influenza epidemic might be coincidence if we didn't have a considerable amount of other evidence to support a definite relation. I think of meningococcal infections as another example of bacterial infection spread by the respiratory tract. It has its own characteristic pattern. It recurs in seasonal waves over a period of five or six years, in a wholly different manner from pneumococcal pneumonia, and with no relation to influenza epidemics. I think of streptococcal infections, what little information we have of their incidence is measured by scarlet fever. Scarlet fever is a disease which occurs more frequently in the late winter and spring. In certain areas—apparently unpredictably, maybe every five, ten or twenty years—there is a rise with a rate four, five, eight, or ten times as high as in the preceding years, lasting for two or three years. Then it declines. This occurred in Baltimore and Washington during the war period, and the rates are now returning to a low level. The epidemiological patterns of bacterial respiratory diseases are each distinct and different. Only the pattern of pneumococcal pneumonia seems to bear a partial relation to epidemic influenza. I do not believe coincidence is an adequate explanation of the epidemiological observations.

Dr. Rich: What is the frequency with which influenza antibody is found in the population in a non-influenza period?

Dr. Langmuir: It is a span. It is a broad distribution from none to a very high

titer. This was shown at Fort Bragg before the 1943 epidemic of type A. Samples were taken from normal soldiers from every area of the country. There had been no epidemic since 1940–41. According to our laboratory technique, about 38 per cent of the bloods titered 1 to 16 or less; about 40 per cent titered 1:32; and 20 per cent 1:64 or higher, something in that range. After the epidemic we again, through the spring of 1944, collected bloods for a variety of reasons, and some of those were tested. A much higher level of antibodies was found. Forty to 50 per cent of specimens had titers 1 to 64 or more. Apparently the antibody level in the whole country was raised. There is no exact figure as to the number of individuals with antibodies in the general population. It is rather a graded scale which varies in relation to epidemics.

Dr. Ward: Dr. Rich's question regarding the sputum from cases other than pneumonia reminds me of Mrs. Maxwell's thesis which was written on this subject. One section of that thesis is called "Defects of the Study." She points out effectively, it seems to me, that what we really need in this particular study are two or three types of answer—the first, how much influenza virus is there among individuals who are well during an influenza epidemic? That question is unknown and cannot be answered; the second, how many cases of diseases other than pneumonia may show virus, is equally unknown. The autopsy material does indicate that there isn't as much as one might suppose. Twenty-six of the lungs examined were from cases occurring during the influenza epidemic and none showed influenza virus. We were able to test the nasal washings of 18 contacts of influenza and secured blood specimens from these individuals. These contacts were interns, assistant residents and nurses on the wards where influenza cases were hospitalized. From two of those individuals we secured virus. A positive serologic response occurred in one of those two. One of the questions Dr. Fred Bang mentioned a few months ago in a similar seminar across the street bears along with Dr. Rich's and Dr. Langmuir's question. How do you know if you tested you could not find other viruses, such as herpes? I don't know—possibly you might. To follow that along another step, if we had some technique for measuring viruses which we think now produce mild respiratory diseases could we show that pneumococcal pneumonia was associated with these particular viruses in a certain proportion of cases?

With regard to Dr. Rich's comment about mitral stenosis playing a part in the death of the second case: I think it is of interest that Dr. Taylor, in 1940, showed that if mice were infected with a sublethal dose of influenza virus and the virus was allowed to grow in the lungs for a couple of days, and then the lung was embarrassed either by inoculation of sterile fluid or simply reanesthetizing the mice, death was produced in the mice. He went on to postulate that what may be occurring in complex or mixed infections of influenza virus and pneumococci or other bacterial agent in man may be that the virus growing inside the cell produces no particular damage at that particular moment, but when the bacteria produce the edema fluid the virus is spread from one cell to another because the cell itself is embarrassed. Here is a patient with mitral stenosis. There is more fluid present

than in a patient who doesn't have mitral stenosis. We have a virus in the lung. That virus, therefore, following Dr. Taylor's thesis along, may be able to spread simply because of the edema fluid in the lung as the cells are "embarrassed" by that edema fluid.

In response to the aspiration question, I think it entirely possible that influenza virus may be aspirated into the lungs just before death. In one of the cases presented tonight, E.T., the lungs titer 10^{-9} in chick embryos. I believe such a titer too high to be explained on the basis of aspiration alone.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal)

Internal Medicine in General Practice, 2nd edition. By ROBERT P. MCCOMBS Illus. 741 pp. $8.00. *W. B. Saunders Company, Philadelphia, Pennsylvania,* 1947.

The doctor with a large general practice is the busiest man in medicine. He must, of necessity, see a great number of patients during the course of each day and seldom has time to sit and ponder over his problems. Frequently these are not great, but in many instances he sees the beginnings of serious disease and more likely than not he must follow these through. He must then be able to recognize them quickly and be competent to treat them. On the other hand, he seldom has the time for extensive reading on new subjects or even for review of the fundamentals of diagnosis. For such a person this book cannot be too highly recommended. It is short, very much to the point, and clearly and interestingly written. Stress is laid entirely on diagnosis and treatment, and controversial points are bridged over or left to the specialist. Many of the rarer diseases are only mentioned in passing, but all the fundamental ones are stressed. Especially helpful are the frequent summaries of differential diagnosis. These are extremely well done and very helpful in the rapid diagnosis which must often be made. There are also excellent summaries of the various important laboratory tests and aids with emphasis on the worth and limitations of each.

Finally, there are the sections on therapy. In this second edition, these have been brought up to date with the addition of the antibiotics and other newer drugs. In addition to covering these, the author has done an excellent job of "debunking" many of the drugs and compounds all too often and too freely used because of the persuasive literature received in the mail. This has been especially well done in the realm of vitamins and hormones.

In conclusion, this is a book which should be in every general practitioner's library. By reading it he can, in a relatively short time, review the fundamentals of internal medicine and at the same time become familiar with the newer concepts of diagnosis and treatment.

W. B. B.

Selected Writings of Benjamin Rush, The. Edited by DAGOBERT D. RUNES. 433 pp. $5.00. *Philosophical Library, New York, New York,* 1947.

A selection from the writings of so many-sided a man as Benjamin Rush is a promising undertaking. The editor has grouped about forty of Rush's lectures, pamphlets, essays, letters, etc., together under the main headings of "On Good

Government," "On Education," "On Natural and Medical Sciences," "On Miscellaneous Things." The section on natural and medical sciences is the longest one and includes, among other things, sections on psychiatry, a field in which Rush's fame has been most enduring. But even those chiefly interested in the physician Rush will welcome this opportunity of making the acquaintance of Rush the theologian, statesman, and educator. For Rush's versatility has made him not only an outstanding figure in the political and civil life of the young and struggling United States, but also one of the most fascinating examples of the "enlightened" doctor.

To the student of Rush, the present selection has the short-coming of giving the date but not the source of the pieces published. An appended list of writings of Rush published during his lifetime is limited to mere titles and can, therefore, hardly be considered a substitute. An additional bibliographical list, however, lists a number of publications which are helpful for further examination.

O. T.

Practical Physiological Chemistry, 12th edition. By PHILIP B. HAWK, BERNARD L. OSER, AND WILLIAM H. SUMMERSON. Illus. 1323 pp. $10.00. *The Blakiston Company, Philadelphia, Pennsylvania*, 1947.

This new edition of the former "Hawk and Bergeim" has the names of Oser and Summerson on the title page. Drs. Hawk and Oser are both associated with Food Research Laboratories at Long Island City. Professor Summerson is a member of the bio-chemistry department of Cornell University Medical College. The preface by Hawk states that this edition "represents a complete revision of the subject matter of this book." He states that "new sections have been introduced on the polarograph, on isotopes, on the sulfa drugs, on metabolic antagonists and antibiotics, on the Warburg tissue slice procedure, on the theory and practice of photometric analysis, on the electrophoretic fractionation of the plasma proteins, on the composition of foods, and on the various vitamins . . . Also many new quantitative procedures for blood and urine analysis have been added."

This volume, like its predecessors, is an excellent book to be kept at the elbow of anyone performing chemical work in a biological field because it provides quick reference to a mass of chemical detail on clinical-biological subjects in a readable form. It provides specific procedures and presents a certain amount of chemical and biological background for them. Considering the complex data in this field, the book provides only rather arbitrary tests and reasons for same. References and background evidence are not emphasized. This means that the significance of the chemical steps or of the biological application must be sought largely elsewhere. It is difficult for the reviewer to make a statement about accuracy except to state that in previous volumes he has found relatively few distinct errors.

F. W. B., Jr.

BOOKS RECEIVED FOR REVIEW

Acute Bacterial Diseases, The. By HARRY F. DOWLING. Illus. 463 pp. $6.50. *W. B. Saunders Company, Philadelphia, Pennsylvania,* 1948.

Care and Managment of Laboratory Animals, The. Edited by ALASTAIR N. WORDEN. Illus. 268 pp. $8.50. *The Williams & Wilkins Company, Baltimore,* 1947.

Clinical Diagnosis by Laboratory Methods. By JAMES C. TODD, ARTHUR H. SANFORD, AND GEORGE G. STILWELL. Illus. 954 pp. $7.50. *W. B. Saunders Company, Philadelphia, Pennsylvania,* 1948.

Dissection of the Cat, The. By BRUCE M. HARRISON. Illus. 109 pp. $3.50. *C. V. Mosby Company, St. Louis, Missouri,* 1948.

Handbook of Treatment and Medical Formulary. By CHARLES M. GRUBER. 594 pp. $7.00. *F. A. Davis Company, Philadelphia 3, Pennsylvania,* 1948.

Human Physiology, 3rd edition. By F. R. WINTON AND L. E. BAYLISS. Illus. 592 pp. $7.00. *The Blakiston Company, Philadelphia 5, Pennsylvania,* 1948

Identification of Tumors. By N. CHANDLER FOOT. Illus. 397 pp. $6.00. *J. B. Lippincott Company, Philadelphia, Pennsylvania,* 1948.

Noah Webster: Letters on Yellow Fever Addressed to Dr. William Currie. 110 pp. $2.00. *The Johns Hopkins Press, Baltimore, Maryland,* 1947.

Treatment by Diet, 5th edition. By CLIFFORD J. BARBORKA. Illus. 784 pp. $10.00. *J. B. Lippincott Company, Philadelphia, Pennsylvania,* 1948.